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CALCIFICATION OF THE MYOCARDIUM

A Pathologic Study of Thirteen Cases

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AND

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CALCIFICATION of the muscle fibers of the heart is rarely observed, but it is not unusual to see deposits of calcium within large myocardial scars. Brown and Evans,¹ in a review of the literature before 1940, were able to find only 12 cases of calcification of myocardial scars, although many pathologists can recall similar instances (unpublished) from their own experience. It was the first radiologic demonstration of an anatomically proved calcified ventricular aneurysm in 1919 which led Scholz² to review the subject thoroughly in 1924. Essentially this review of the material at the Army Institute of Pathology merely confirms his observations on the cases collected from the literature. It was not always clear from his account of the reported cases whether or not the mineral deposit had involved muscle fibers themselves or the scar tissues which had replaced necrotic foci. This distinction has been drawn in the present series and an effort has been made to ascertain, if possible, the factors responsible for it.

Our attention was first called to calcification of the myocardium by an interesting case, briefly outlined here but presented in detail in another context.³ While running an obstacle course, a white soldier, 23 years of age, struck his chest forcibly against a wall and was thrown to the ground. Though he completed the run, he staggered and suffered from precordial oppression. He was hospitalized promptly in a state of severe shock. There was ventricular tachycardia of 264 per minute. Therapeutic measures directed at ending both the shock and the tachycardia were not effective for twenty-six hours. Subsequently, severe and stubborn oliguria set in, which persisted until death, seven days later, caused by uremia and left ventricular failure. During the period of hospitalization, repeated electrocardiograms had shown evidence of

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1 Brown, C E, and Evans, W D. *Am Heart J* **19** 106, 1940

2 Scholz, T. *Arch Int Med* **34** 32, 1924

3 Gore, I. *The Question of Traumatic Heart Disease*, to be published

severe myocardial damage, clinically a gallop rhythm had been observed from the fourth day onward

Since the patient had appeared to be in good health, had fulfilled the rigid physical requirements for Officer Candidate School and had experienced no difficulty from participation in strenuous activity, the heart disease was regarded as traumatic⁴ However, pathologic examination promptly excluded that possibility, revealing myocarditis which clearly had preceded the traumatic incident There were large macroscopic areas of myocardial necrosis, under the microscope many of the

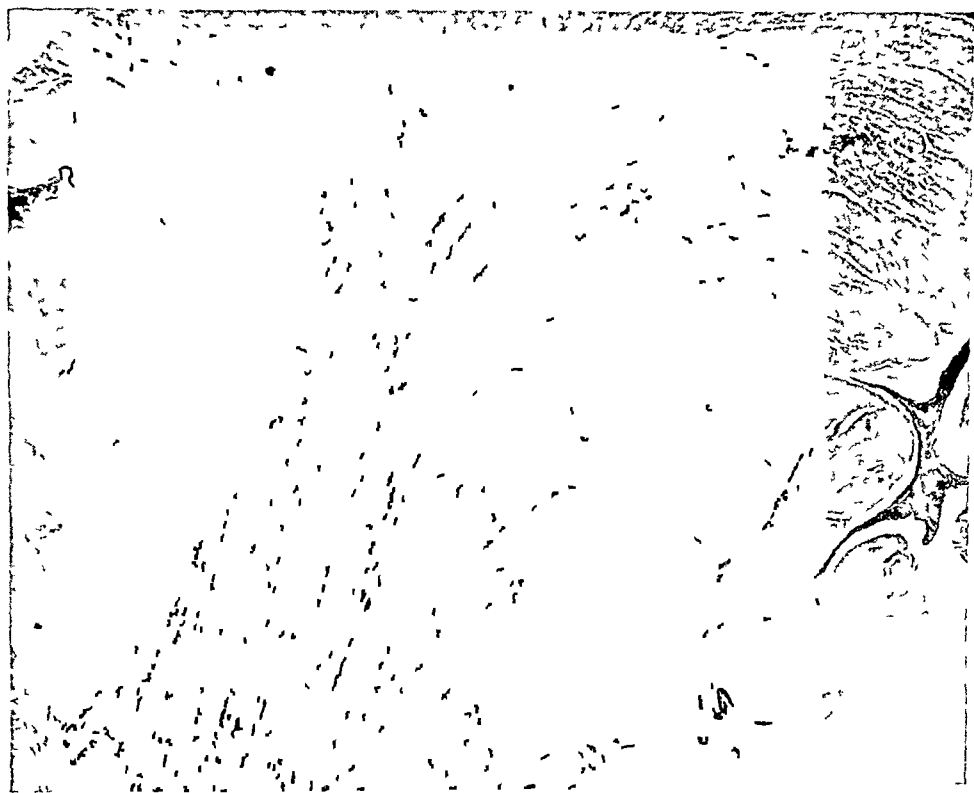


Fig 1 (case 2, AIP Neg 75716) —Microtessar photograph of a section through the posterior part of the interventricular septum The darkly stained areas represent deposits of calcium in degenerated portions of the myocardium Hematoxylin and eosin, $\times 10$

involved muscle fibers were heavily incrustated with calcium, verified by the von Kossa stain (fig 1) Such areas had imparted a gritty sensation to the knife blade In interpreting this unusual finding, it seemed reasonable to assume that the renal failure, a result of lower nephron nephrosis, had facilitated deposition of calcium on initially necrotic heart muscle A review of material with similar deposits within the myocar-

⁴ Warburg, E Brit Heart J 2 271, 1940 Arenberg, H Ann Int Med 19 326, 1943

dium was undertaken at the Army Institute of Pathology in order to collect evidence by which the validity of the premise formulated in this one case could be evaluated

MATERIAL AND METHODS

Records and tissues in which the reviewing pathologist had noted the presence of calcific deposits within the myocardium were available from 13 autopsies. In addition to the slides routinely stained with hematoxylin and eosin, selected sections were prepared by the von Kossa technic for calcium, and the Turnbull and prussian blue reactions for iron. Stains for iron were done because of the intimate relation between iron and calcific deposits which is so frequently cited, and also in order to investigate the possibility that the breakdown of myoglobin, specifically, might be a contributory factor in causing the deposition of calcium.

OBSERVATIONS

The results of this investigation together with other pertinent data are listed briefly in the accompanying table. Case 2 (AIP Acc 88679) will be recognized as the one just described.

The one factor common to all cases was the presence of a pathologic process leading to myocardial necrosis. Calcification was never observed in the absence of necrosis of muscle, but a varying quantity of uncalcified degenerated muscle could be demonstrated in every case. The causes of the degenerative myocardial process were ischemia in 4 cases (9, 10, 11 and 12), myocarditis of infectious origin in 3 cases (1, 4 and 5) and a combination of ischemic and infectious factors in 2 cases (3 and 9). In 4 cases (2, 6, 7 and 13) the cause of the degenerative myocardial process was unknown. The duration of the terminal illness, a clinical figure, not necessarily an accurate measure of the predisposing process, varied from one day to eleven months, but in each instance the degenerative process in the heart, judged histologically, appeared to be relatively recent, measurable in days or, at most, weeks.

The reactions with the special stains demonstrated the fallacy of a practice common among pathologists, i.e., the identifying of an incrustation or deposit as calcium from the deep purple color which it assumes in hematoxylin and eosin preparations. In 6 cases both iron and calcium were demonstrated (figs 1, 2, 3 and 4), in 4 the entire deposit proved to be calcium (fig 5) and in 3 iron only was found (fig 6). The two deposits were not always coextensive, and consequently a clearcut relation between them could not be established.

The kidneys were sufficiently diseased in 11 cases to cause significant functional impairment, demonstrated clinically by blood nitrogen retention in 8. The renal involvement was of diverse pathologic types. There were 4 examples of lower nephron nephrosis (cases 1, 2, 4 and 6), 4 of severe arteriolar nephrosclerosis (cases 8, 9, 10 and 12), 2 of acute and subacute glomerular nephritis (cases 7 and 13) and 1 of severe focal embolic glomerular disease (case 3). Azotemia of prerenal origin could be reasonably assumed in a twelfth case (5), that of a patient who died in congestive heart failure as a result of diphtheritic myocarditis. The sole patient in whom there was no reason to suspect renal excretory inadequacy was an infant of 4 months (case 11) with a history of excessive intake of vitamin D over a period of two and one-half months.

Foci of calcification outside the heart were observed in 5 cases. Chalky deposits were associated with acute pancreatic fat necrosis in case 8, in case 6 necrotic cells in a traumatic infarct of the liver were incrustated, focal calcification of the interalveolar septums of the lungs was present in cases 7, 11 and 12, and in

Case	AIP Account Number	Age, Yr, Race, Sex	Nature of the Myocardial Lesion	Minerals Demonstrated Histologically		Factors of Etiologic Significance in the Production of the Myocardial Lesion	Aortic Lesion	Cause of Azotemia	Blood Chemistry, Mg per 100 Cc	Associated Pathologic Changes	Duration of Terminal Illness
				Calcium	Iron						
1	86,202	38 N M	Mineralization involves muscle fibers in widespread foci of acute degeneration there is a mild reactive mononuclear cell infiltration	+	+	Lobar pneumonia	+	Lower nephron nephrosis (sulfa effect)	Nonprotein nitrogen, 400 Creatinine, 7.3	Inactive syphilitic aortitis, focal hepatic necroses, terminal pneumococcal sepsis	9 days
2	88,679	23 W M	Acute myocarditis, widespread muscle degeneration with a prominent infiltrate of granulocytes and mononuclear cells, extensive mineralization of necrotic fibers	+	±	Unknown Histologically, the cardiac lesion clearly antedates the acute symptoms which occasioned hospitalization	+	Lower nephron nephrosis	Nonprotein nitrogen, 186 Phosphorus, 8.8 Calcium, 9.7	Central zonal necrosis of the liver	? Death occurred on the 9th hospital day
3	91,096	21 N F	Widespread foci of ischemic necrosis, old and recent mineral incrustation has involved many of the most recently damaged fibers	—	—	Subacute bacterial endocarditis involving the mitral valve	+	Multiple renal infarcts, focal embolic glomerulonephritis	None	Infarcts of lung and spleen	6 mo
4	92,261	43 W M	Small foci of acute myocardial necrosis with moderate granulocytic infiltration, mineralization has involved many of the degenerate fibers	+	—	Bronchopneumonia, acute hepatitis	+	Lower nephron nephrosis	Nonprotein nitrogen, 110-210 Creatinine, 16 CO ₂ 61.71 volts %	Acute hepatitis with central zonal necrosis, bronchopneumonia	15 days
5	109,334	26 W M	Widespread foci of muscle necrosis with prominent leukocytic reaction some of the involved fibers show mineral incrustation	—	+	Pharyngeal diphtheria	+	Cardiac failure, prerenal azotemia	None	Congestive heart failure	12 days
6	118,951	21-39 W M	Focal necroses and fibrosis with surrounding mixed leukocytic response, mineralization of many of the acutely necrotic fibers	—	+	Unknown Histologically the cardiac changes easily antedate the terminal clinical episode	+	Lower nephron nephrosis	None	Calcification of necrotic cells in liver infarcts, multiple shell fragmentation of right lower leg	4 days
7	127,902	27 W M	Tiny scattered foci of acute myocardial degeneration with scant mononuclear cell reaction, many of the necrotic fibers are heavily mineralized	+	+	Unknown The cardiac process is much more recent and acute than the renal disease	+	Subacute glomerulonephritis	Nonprotein nitrogen, 132 Calcium, 7.6 CO ₂ , 56 volts %	Bronchopneumonia, focal mineralization of pulmonary interalveolar septums	5 mo
8	144,674	52 W M	Numerous tiny foci of acute necrosis, most of the fibers are heavily incrustated reaction is sparse and predominantly mononuclear, necrotic fibers at the margins of occasional pyemic microabscesses also show mineral deposition	+	±	Bronchopneumonia and terminal pyemia, coronary sclerosis and circulatory failure	+	Severe arteriolar nephroses	Nonprotein nitrogen, 137.25 CO ₂ , 78.84 volts %	Bronchopneumonia, acute pancreatitis with chalky deposits in fat necroses, terminal sepsis with pyemic abscesses of kidney	7 mo
9	183,518	47 W M	Healing myocardial infarct with terminal extension calcification has involved the acutely necrotic muscle though the process is sufficiently recent to be without leukocytic response	+	+	Severe coronary sclerosis and thrombosis of left C.A., cardiac decompensation	+	Severe arteriolar nephroses, circulatory failure	Nonprotein nitrogen, 146 Creatinine, 5.8	Bronchopneumonia, congestive heart failure	Mild decompensation, 3 yr severe decompensation, 6 wk 21 days
10	184,634	61 W M	Old and recent myocardial infarcts acutely necrotic fibers at periphery of infarcts are heavily incrustated intense granulocytic response	+	±	Severe coronary sclerosis, pneumonia and circulatory failure	+	Severe arteriolar nephroses, circulatory failure	Blood urea nitrogen 100 CO ₂ , 45 volts %	Bronchopneumonia, congestive heart failure	1 day
11	187,787	4/12 W M	Foci of ischemic necrosis with mineralization of many of the fibers inflammatory reaction absent	+	—	Coronary thrombosis left C.A. secondary to medial calcification of coronary arteries, hypertensive D (3,400 units daily—3 mo)	—	None	None	Acute congestive heart failure, calcification involving the elastica of the systemic arteries and foci, interalveolar septums of lungs	11 mo
12	188,406	67 W M	Total acute muscle degeneration with prominent mixed type of leukocytic response most of the necrotic fibers are heavily mineralized fairly prominent myolysis indicates duration of at least 1 week	—	+	Moderately severe coronary sclerosis, bronchopneumonia	+	Severe arteriolar nephroses extensive alkalotic nephroses	None	Chronic duodenal ulcer, pyloric obstruction, gastric retention, terminal bronchopneumonia focal calcification of interalveolar septums of lungs	11 mo
13	196,797	37 W M	Conspicuous and numerous but small foci of mineralization superimposed upon necrotic muscle there is only slight associated inflammatory infiltration	+	+	Unknown Histologically the cardiac changes are too recent to correlate with any of the other findings	+	Acute glomerulonephritis	Nonprotein nitrogen, 102.173	Hemochromatosis, portal cirrhosis with liver failure	Cirrhosis, 3 yr Nephritis 12 mo

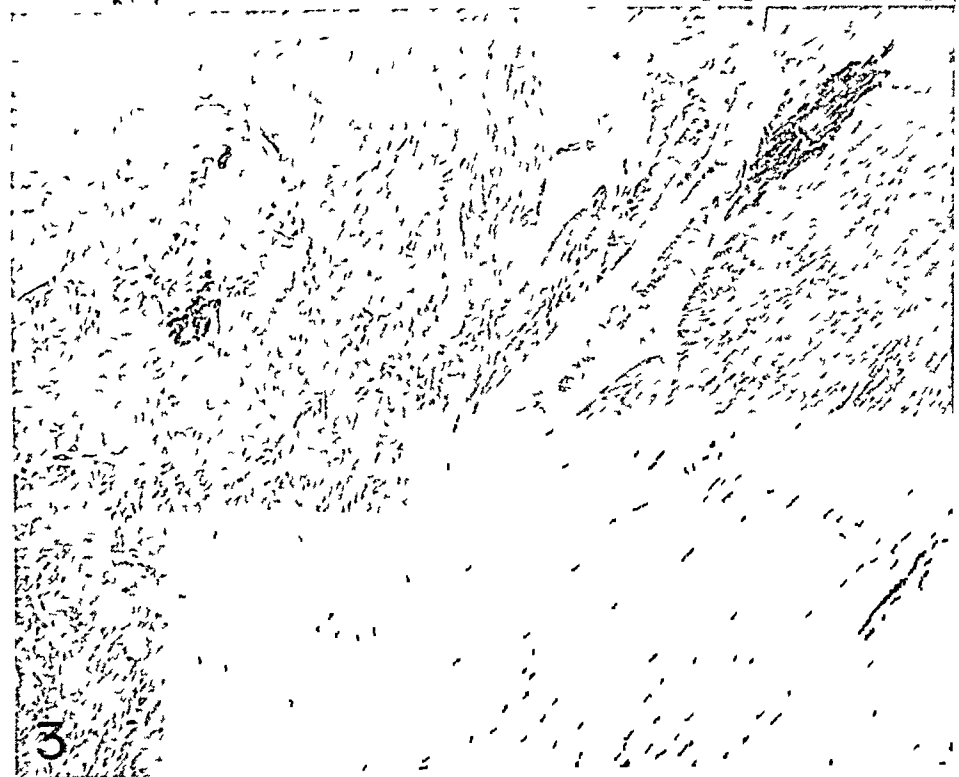
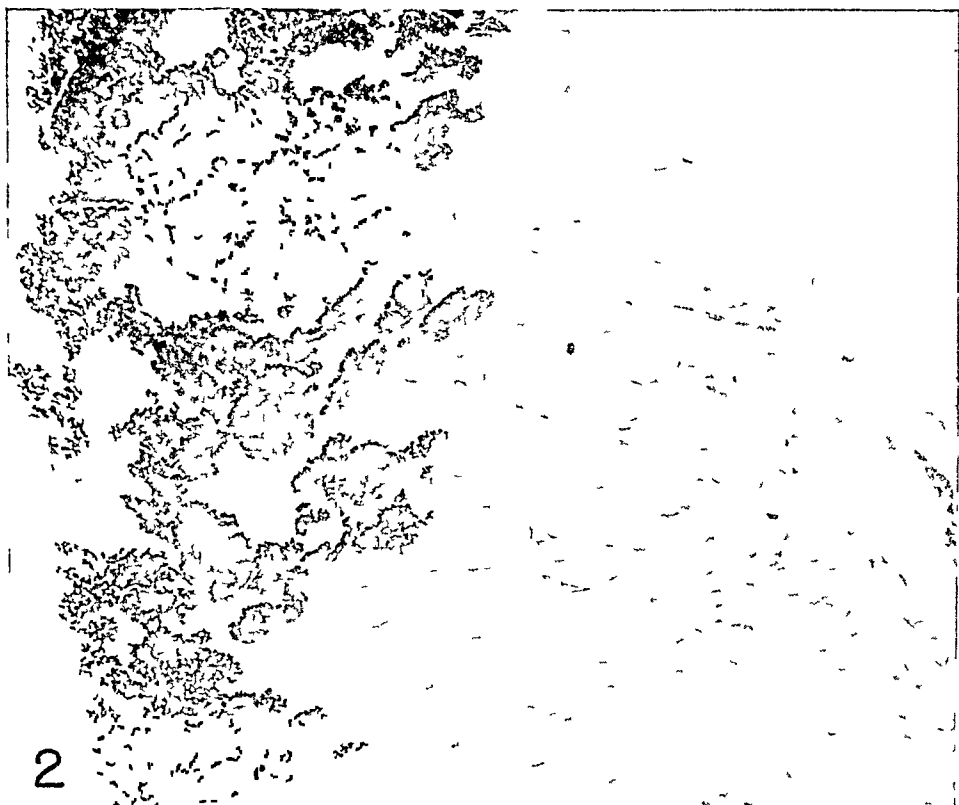


Fig 2 (case 1, AIP Neg 86202) —A portion of the left ventricular wall stained with hematoxylin and eosin. The darkly stained coalescent areas in the outer myocardium represent calcified necrotic muscle fibers. $\times 12$

Fig 3 (case 13, AIP Neg 196797) —Observe the three darkly stained areas representing deposits of calcium in necrotic foci of heart muscle. Hematoxylin and eosin, $\times 28$

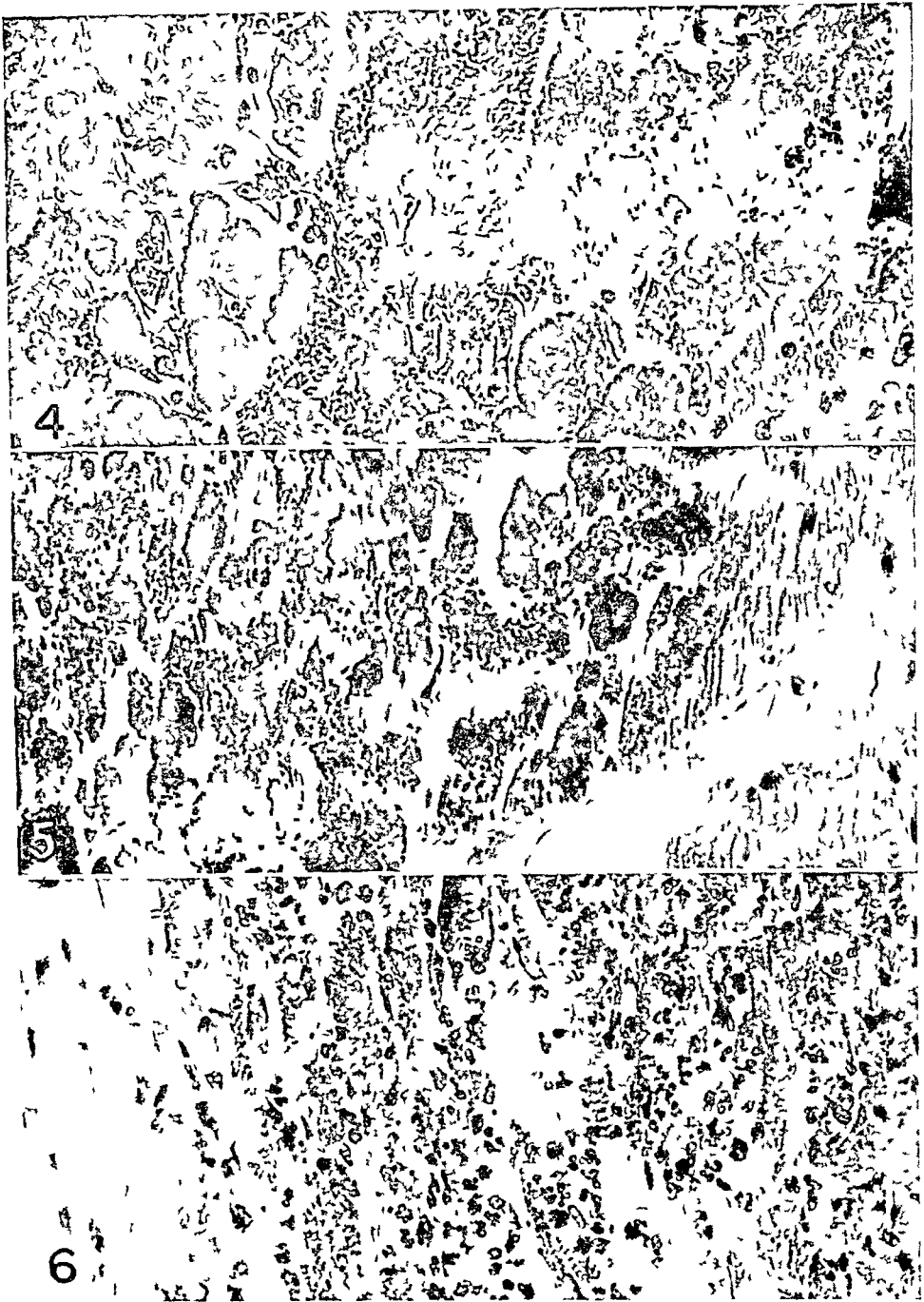


Fig 4 (case 2, AIP Neg 86202) —Deposits of calcium on necrotic myofibrils account for their dark stain and granular character. To the left of the center in the upper portion of the photograph, and to the right in the lower portion, uncalcified necrotic fibers are to be noted. Hematoxylin and eosin, $\times 355$

Fig 5 (case 3, AIP Neg 91096) —Observe that the granular deposits of darkly stained calcium do not involve the necrotic myofibrils uniformly. Hematoxylin and eosin, $\times 355$

Fig 6 (case 5, AIP Neg 109334) —The deposits of darkly stained granular material on the necrotic myofibrils represent iron. The leukocytic reaction provides a clue as to the duration of the degenerative process. Hematoxylin and eosin, $\times 300$

case 11 (that of the infant previously referred to) there was also widespread calcification of the internal elastic lamina of the systemic arteries

Unfortunately, there were only 2 patients in whom blood calcium levels had been determined before death. In case 2 the value was within normal limits (97 mg per hundred cubic centimeters), but the corresponding phosphorus value was significantly elevated to 88 mg. A figure of 76 mg of calcium in case 7 is distinctly low but cannot be properly evaluated without knowledge of the plasma protein level, which is likely to be reduced in subacute glomerular nephritis.

COMMENT

Pathologic calcification is generally considered to be either dystrophic or metastatic in nature,⁵ the former being by far the more frequent. As Wells⁶ has stated, "any area of dead tissue that is not infected and that is so large or so situated that it cannot be absorbed, will probably become infiltrated with lime salts." Calcification is common, particularly in old atheromatous lesions of the aorta, it characterizes the medial sclerosis of peripheral arteries. Deposits of calcium in foci of necrosis occur sufficiently often to be considered of some significance, at least in the roentgenologic diagnosis of such conditions as tuberculosis, histoplasmosis and toxoplasmosis. Hemorrhagic extravasates seem to be particularly susceptible to calcification, and examples often seen clinically are myositis ossificans and the deposits which occur in degenerating and hemorrhagic "adenomas" of the thyroid gland. Precipitation of lime salts in hyaline scars, though necrosis is not involved, is also regarded as a form of dystrophic calcification. It has been related to the low production of carbon dioxide in a slowly metabolizing tissue, which permits development of a local zone of relative alkalinity and, consequently, local reduction of calcium solubility. Dystrophic calcification may proceed without variation of the physiologic levels of any of the chemical constituents of the blood.

On the contrary, metastatic calcification is associated with increased availability of calcium. As Mulligan⁷ has emphasized, this type of mineralization has been found with bone destructive lesions, with hyperparathyroidism and hypervitaminosis D and with renal insufficiency. Elevated levels of blood phosphorus occur in renal insufficiency, calcium may remain unchanged, as in case 2, or may even be depressed, as in case 7. However, secondary parathyroid hyperplasia and hyperactivity associated with chronic renal disease may, at times, result in hypercalcemia. Metastatic calcification, unlike the dystrophic type, has a characteristic distribution.⁵ The chemical structure of elastic tissue serves

5 Karsner, H. T. *Human Pathology*, ed. 6, Philadelphia, J. B. Lippincott Company, 1942.

6 Wells, H. G. *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

7 Mulligan, R. M. *Arch. Path.* **43**: 177, 1947.

to explain involvement of arterial and endocardial (right auricle) elastica by metastatic lime salt deposits⁸ In the lungs the veins rather than the arteries are involved,⁹ indicating the influence that high oxygen and low carbon dioxide tensions exert through their effect on the hydrogen ion concentration Wells provided the explanation, since generally accepted, for the localizing of metastatic mineral deposits in and about such tissues as pulmonary alveolar septums, gastric mucosa and renal tubular epithelium The physiologic activity of these structures results in local alkalinity of tissue by virtue of the excretion or secretion of acid¹⁰ Confirming the importance of an alkaline reaction in permitting or facilitating lime deposits, Mulligan and Stricker¹¹ found that lesions were more severe in dogs receiving alkaline salts to complement the effect of excessive doses of vitamin D which were used to produce metastatic calcification Though heavily mineralized tissues are obviously incapable of the complex chemical and physical interchanges essential to life, it seems likely that the initial deposition occurs on vital tissues and constitutes still another distinction from dystrophic calcification¹² The selective localization of metastatic deposits of lime salts is, therefore, a function of both normal structure and physiologic activity in the presence of systemically increased availability of calcium, dystrophic calcification owes its occurrence purely to local pathologic changes and has no characteristic distribution

A brief discussion of the chemical basis of pathologic calcification seems warranted Normally the blood level of calcium represents chemical saturation¹³ The relation of calcium to phosphate is of prime importance to the occurrence of either ossification or calcification since, as has been amply verified, the chemical bulk of such deposits consists of the insoluble form of tricalcium phosphate¹⁴ In such a saturated solution of a poorly soluble substance, the relations of the concentrations of ionic calcium and phosphate, molecular calcium phosphate and the undissolved salt, following the laws of ionic equilibrium, explain the reciprocal alterations usually observed in the blood levels of these two substances¹⁵ An excess of calcium or of phosphate or of both, such as occurs in conditions producing metastatic calcification, shifts the equilibrium toward the formation of more insoluble salt Decreasing hydrogen ion concentration causes a similar shift in the equilibrium, since there is decreased solubility of molecular calcium phosphate Dystrophic calcification, of course,

8 Hass, G M Arch Path **27** 334 and 583, 1939

9 Wells⁶ Karsner⁵ Mulligan⁷

10 Wells⁶ Mulligan⁷

11 Mulligan, R M, and Stricker, F L Am J Path **24** 451, 1948

12 (a) Barr, D P Physiol Rev **12** 592, 1932 (b) Scholz² (c) Wells⁶

13 (a) Logan, M A Physiol, Rev **20** 522, 1940 (b) Barr^{12a}

14 Wells⁶ Logan^{13a}

15 Howland, J, and Kramer, B Tr Am Pediat Soc **34** 204, 1922 Logan^{13a}

obeys the same natural laws, but in a manner less easily explained. Superficially it would seem that the elevation of hydrogen ion concentration which accompanies necrosis would prevent the deposition of lime salts, and perhaps this is one of the reasons dystrophic calcification is not more common than it is. However, the enzymic degradation of tissues, with their content of nucleotides, phospholipids and phosphoric esters, results in a local excess of phosphate which could easily cause calcium to be precipitated from its physiologic levels. Rapid absorption of such products understandably precludes that possibility. The activity of phosphatase, which is regarded as significant in ossification, does not appear to be essential to the explanation of pathologic calcification. In discussing this problem, Wells⁶ had questioned that the phosphoric acid contents of tissues have any relation to the frequency with which they exhibit calcific deposits. He had found experimentally that boiled tissues rich in nucleoprotein and phosphoric acid implanted into the abdominal cavity of rabbits showed no more extensive calcification than did tissues poor in these substances. This observation may be interpreted equally well as evidence that the potential phosphate content of any tissue is adequate to produce deposition of calcium by this mechanism and that the additional quantities present in tissues rich in nucleoprotein are superfluous, provided that the blood calcium remains at a physiologic level. It is obvious that any condition favoring the development of metastatic calcification would, by increasing the availability of calcium, simultaneously favor and accelerate the dystrophic variety.

Inasmuch as necrosis was invariably present and seemed to be the *sine qua non* of myocardial calcification in these cases, the process must be considered dystrophic. However, in case 11, an instance of vitamin D intoxication, autopsy showed, in addition, typical metastatic calcification of the systemic arteries and lungs, and in the remaining cases, without exception, there was evidence of renal excretory inadequacy and azotemia. It is interesting that extensive renal disease was also a factor in many of the cases collected by Scholz.² He noted that the lesions of the kidneys were correlated with a degenerative process of varied causation in the heart, but his explanation, limited by the primitive knowledge of calcium and phosphorus metabolism, would be regarded as inadequate today. As Mulligan⁷ has so capably outlined, the changes of calcium and phosphate balance produced by renal failure favor the metastatic deposition of lime salts. As a matter of fact, such foci were present in the lungs in 2 of our cases (7 and 12). Failure to demonstrate them in the other cases of this series is not surprising, for, to judge from the examples observed, the formation of these lesions is usually gradual. The point remains that in these cases necrotic tissues were exposed to conditions which favored and accelerated the deposition of

lime salts Necrotic heart muscle and its products are ordinarily absorbed too rapidly to permit calcification In diphtheritic myocarditis, Gore¹⁶ has shown that myolysis, which begins early in the second week, is fairly complete by the end of the third week The resorptive process may be somewhat delayed in the central part of large infarcts, relatively remote from an effective blood supply, but access of calcium salts is limited for the same reason, this is illustrated in case 10, in which a heavier incrustation occurs at the periphery of the infarct In infants, however, in whom there is a physiologic elevation of blood phosphorus, and in small experimental animals with active metabolism it is not uncommon to see calcific deposits in areas of myocardial necrosis Kaunitz,¹⁷ reporting on an anomalous origin of the left coronary artery, gave an example of myocardial calcification occurring in an infant and listed several other cases in which the fundamental lesion was ischemic The virus-induced myocardial changes produced by Pearce¹⁸ in rabbits were calcified within a few days after the introduction of the causative infectious agent

Perhaps because clinical evidence of calcification is slow to appear in such diseases as pulmonary tuberculosis, the rapidity with which it sometimes takes place is not generally appreciated In the experimental work of Pearce¹⁸ it occurred within a few days, though no condition was described which might have caused an acceleration In case 6 of this series, liver cells which could not have been necrotic longer than four days were already heavily calcified In the other cases the time relations were not so clearly defined, but the failure to demonstrate significant myolysis in the involved degenerated areas of the heart indicated the recency of the necrosis, which of necessity had preceded the deposition of mineral salt In case 8, for example, many of the necrotic and secondarily calcified foci were related to pyemia which developed terminally Wells⁶ observed deposition of mineral salts as soon as four hours after the occurrence of pancreatic fat necrosis, but in that condition the precipitate was formed of insoluble calcium soaps rather than phosphates

The nature of the processes leading to myocardial necrosis needs little comment The relationship of infectious diseases and of certain drugs (sulfonamides, in particular) to myocarditis has been adequately covered in the literature¹⁹ Ischemia as a cause of cardiac degeneration requires no documentation Yet, even when all the factors have been considered, there still remain 4 of our cases in which the pathogenesis

16 Gore, I *Am J M Sc* **215** 257, 1948

17 Kaunitz, P E *Am Heart J* **33** 182, 1947

18 Pearce, J M *Arch Path* **34** 319, 1942

19 Gore, I *Am Pract* **1** 292, 1947 Gore, I, and Saphir, O *Am Heart J* **34** 827, 1947 Saphir, O *Arch Path* **32** 1000, 1941, **33** 88, 1942

of the cardiac lesions was obscure. In 2 cases (7 and 13) of subacute and glomerular nephritis, respectively, the type of myocardial change was identical—multiple, scattered tiny foci of acute necrosis which obviously must have occurred late in the course of the rather prolonged terminal illness (fig. 3). The lesions are to be distinguished from the serious type of myocarditis which may complicate the course of acute or subacute glomerular nephritis.²⁰ They appear to be similar to the miliary necroses and foci of interstitial inflammation which Solomon, Roberts and Lisa²¹ described in 25 of the hearts of 26 uremic patients who died of circulatory or congestive heart failure. Experimentally, Smadel and Farr²² have observed the lesions of acute myocardial necrosis in 14 of 20 rats with nephrotic nephritis and protracted renal insufficiency. Certain rather characteristic electrocardiographic disturbances in uremia have been related clinically to potassium intoxication, but in the cases studied clinically and at autopsy by Langendorf and Pirani²³ focal myocardial necroses were not observed. There is the possibility that in uremia the accumulation of nonvolatile metabolites eventually reaches a point at which it becomes incompatible with the survival of a metabolically highly active and unresting tissue. Although the available information is inadequate as yet to restate this premise in specific chemical terms, it is a thesis for which the well proved laws of mass action and chemical ionic equilibrium demand support. The focal rather than general distribution of lesions produced in this fashion may be accounted for by minor differences of the cells within the tissue and by slight variations in their relations to the circulation. If we may be permitted to draw an analogy, Himsworth²⁴ has summarized the data which relate certain focal lesions of the liver to variations in the distribution of the blood. Systemic conditions, such as hyperpyrexia,²⁵ thiamine deficiency²⁶ and lack of potassium²⁷ have been shown to produce lesions of focal distribution in the heart.

We were unable to draw any conclusions regarding the relationship of calcium and iron deposition. Hektoen²⁸ was among the first to call attention to the tendency of calcium-iron incrustation to involve elastic

20 Gore, I, and Saphir, O. *Am Heart J* **36** 390, 1948

21 Solomon, C, Roberts, J E, and Lisa, V R. *Am J Path* **18** 729, 1942

22 Smadel, J E, and Farr, L E. *Am J Path* **15** 199, 1939

23 Langendorf, R, and Pirani, C L. *Am Heart J* **33** 282, 1947

24 Himsworth, H P. *The Liver and Its Diseases*, Cambridge, Mass., Harvard University Press, 1947

25 Gore, I, and Isaacson, N. *The Pathology of Hyperpyrexia*, *Am J Path*, to be published

26 Rinehart, J F, Greenberg, L D, and Friedman, M. *Am J Path* **23** 879, 1947

27 Folliis, R H, Jr. *The Pathology of Nutritional Disease*, Springfield, Ill., Charles C Thomas, Publisher, 1948

28 Hektoen, L. *J M Research* **7** 159, 1902

fibers in the vicinity of hemorrhages Klotz²⁹ showed that the siderofibrotic nodules which occur in the spleen in splenic anemia often become calcified The frequency with which either deposit occurred alone in the present material and their failure to be completely coextensive when both were present carry no conviction of an intimate association Possibly the short duration of the process accounts for this variation from changes usually described in much older lesions Certainly, those instances in which iron was not demonstrable offer proof that the breakdown of myoglobin in the areas of cardiac necrosis was not essential to the subsequent deposition of calcium Cameron³⁰ has presented excellent reasons for doubting the specificity of the von Kossa reaction for calcium but has also noted the inconstancy of the association of iron and calcium in normal as well as in pathologic mineral deposits

SUMMARY

In 13 cases of calcification of the myocardium at the Army Institute of Pathology, calcium deposits had been laid down on necrotic muscle fibers which ordinarily are absorbed too rapidly to permit this eventuality However, under circumstances which favor metastatic calcification, there is an augmented and accelerated tendency toward the development of dystrophic calcification The process in the heart muscle in these cases is therefore regarded as an example of accelerated dystrophic mineralization In 1 case there was a history of excessive intake of vitamin D, and at autopsy there were, in addition to the cardiac lesions, widespread metastatic deposits of calcium Azotemia occurred in all 12 of the remaining cases, being of renal origin in 11 and of prerenal origin in 1 The pathologic types of renal involvement included both glomerular and vascular renal disease, as well as lower nephron nephrosis The laws of mass action and of ionic equilibrium of saturated solutions of poorly soluble salts explain, in the case of tricalcium phosphate, the ease with which precipitation may be induced by increases of either calcium or phosphate

The pathogenesis of the basic myocardial lesion also varied, and in the cases studied could be related to ischemia, to an infectious process, to combinations of the two and to unknown influences The possible influence of uremia in causing multiple tiny foci of myocardial necrosis in 2 cases was considered

Special staining reactions for iron and calcium showed the fallacy of making a diagnosis of calcification from a preparation stained with hematoxylin and eosin In 3 instances the deposit proved to be iron only Calcium was present in all the others, in 6 it was associated, but not always coextensive, with iron

29 Klotz, O Bull Johns Hopkins Hosp 27 363, 1916

30 Cameron, G R J Path & Bact 33 929, 1930

LIPID FRACTIONS OF HUMAN ADRENAL GLANDS

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ADRENAL lipids, subject to repeated study since Virchow¹ first noted their rich concentration, have recently acquired new interest from the evidence that cholesterol is a precursor of the corticosteroid hormones and that fluctuations in adrenal cholesterol may reflect secretory activity² Early reports of variations in adrenal lipid detected by morphologic means described reduction of the anisotropic lipid of human adrenal glands in acute and chronic infection³ and comparatively high levels of adrenal lipid in advanced arteriosclerosis, chronic nephritis and cerebral hemorrhage⁴ Similar conclusions resulted from early chemical studies, in which relatively high values of adrenal cholesterol were found in hypertension⁵ and varied types of cardiovascular renal disease,⁶ while septicemia and other severe infections were associated with the lowest comparative values for adrenal cholesterol

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1 Virchow, R Virchows Arch f path Anat **12** 484, 1857

2 (a) Long, C N H, in Pincus, G Recent Progress in Hormone Research, New York, Academic Press, Inc, 1946, vol 1, p 99 (b) Sayers, G, Sayers, M, White, A, and Long, C N H Proc Soc Exper Biol & Med **52** 200, 1943, (c) Yale J Biol & Med **16** 361, 1944

3 (a) Borberg, N C Skandinav Arch f Physiol **32** 287, 1915 (b) Herrmann, O Ueber Vorkommen und Veranderungen von Myelinsubstanzen in der Nebenniere, Arb a d. Geb d path Anat **5** 419, 1906 (c) Kawamura, O Die Cholesterinverfettung, Jena, G Fischer, 1911, cited by Weltmann^{3d} (d) Weltmann, O Beitr z path Anat u z allg Path **56** 278, 1913

4 (a) Knack, A V Virchows Arch f path Anat **220** 36, 1915 (b) Borberg^{3a} (c) Weltmann^{3d}

5 Chauffard, A, Laroche, G, and Grigaut, A (a) Compt rend Soc de biol **73** 23, 1912, (b) **76** 529, 1914

6 (a) Fex, J Biochem Ztschr **104** 82, 1920 (b) Landau, M, and McNee, J W Beitr z path Anat u z allg Path **58** 667, 1914 (c) Wacker, L, and Hueck, W Arch f exper Path u Pharmakol **71** 373, 1913 (d) Chauffard and others⁵

In several instances the data reveal that analyses of adrenal glands of normal persons yielded lipid values in high ranges, comparable to those in persons suffering from hypertension and nephrosclerosis⁷

More recent chemical studies of human adrenal lipids have indicated an inverse relationship between phospholipid and histologically demonstrable fat⁸ as well as between water and lipid content^{8b}. Another report claimed no simple relationship between phosphatides and total cholesterol⁹. The relatively high values of adrenal lipid and cholesterol observed in circulatory diseases, including hypertension, and the low values in infection have been confirmed¹⁰ as in earlier studies. Hyperthermia per se has been asserted to correlate with reduction of adrenal cholesterol¹¹ but was actually not separable as a factor from the infection present in most instances.

In the few recent series of cases in which lipid analyses were performed on normal human adrenal glands (death was caused by violence) the small number of values reported for total lipid^{8b} and cholesterol¹² have been among the highest in each series, again comparable with those found in cases of hypertension and of cardiovascular and renal disease.

In a single report^{10b} nonsterol fat was said to have been found in higher total quantity in the adrenal glands of hypertensive patients than in those of normal persons, although the total amounts of ester and free cholesterol in the two groups were comparable. Lipid concentration, determined both chemically and histologically, was found to correlate well with the presence of hypertension in a recent study¹³ in which the control series consisted not of normal persons but of a group of nonhypertensive patients dead of miscellaneous disorders.

In most reports¹⁴ hypertension and other types of circulatory disease not necessarily accompanied by hypertension were similarly associated with high values of adrenal lipid and cholesterol. In a recent morphologic study of adrenal cortical lipids,¹⁵ however, dis-

7 Weltmann^{8d} Fex^{6a} Landau and McNee^{6b}

8 (a) Kutschera-Aichbergen, H. *Verhandl d deutsch path Gesellsch* **20** 133, 1925 (b) Materna, A., and Januschke, E. *Virchows Arch f path Anat* **263** 537, 1927

9 Woodhouse, D. L. *Biochem J* **22** 1087, 1928

10 (a) Koch, K., and Westphal, K. *Deutsches Arch f klin Med* **181** 413, 1937 (b) Liebegott, G. *Beitr z path Anat u z allg Path* **109** 93, 1944 (c) Kutschera-Aichbergen^{8a} (d) Materna and Januschke^{8b} (e) Woodhouse⁹

11 Ewert, B. *Upsala lakaref forhandl* **40** 423, 1934

12 Koch and Westphal^{10a} Liebegott^{10b} Ewert¹¹

13 Fisher, J. A., and Hewer, T. F. *J Path & Bact* **70** 605, 1947

14 Fex^{6a} Landau and McNee^{6b} Wacker and Hueck^{6c} Materna and Januschke^{8b} Koch and Westphal^{10a} Ewert¹¹

15 Sarason, E. L. *Arch Int Med* **71** 702, 1934

tinctly greater quantities of stainable fat were reported observed in the adrenal glands of persons who died of hypertension than in those of patients with marked arteriosclerosis without hypertension or in those of a small group of normal adults

Although a survey of previously determined values of the major lipid components of human adrenal glands is of interest, the variety of technics, the conditions of collection and the methods of expressing

TABLE 1—Summary of Previously Published Values of Total Lipid and Cholesterol Concentrations of Human Adrenal Glands

Author	Cases	Type of Cases	Mean Values and Ranges		Expressed as
			Total Lipid	Cholesterol	
Fisher and Hewer ^{1*}	127	Hypertension and miscellaneous nonhypertensive diseases	7.2		Per cent wet weight of adrenal tissue
Liebegott ^{10b}	7	Normal	16.1	4.9	Per cent wet weight of a single adrenal gland
	10	Hypertension, cardiac compensation	12.5	3.1	
	9	Hypertension, cardiac decompensation	10.6	3.6	
	6	Nonhypertensive condition, cardiac decompensation	6.1	3.1	
	41	Acute and chronic infections	5.7	1.1	
Koch and Westphal ^{10a}	9	Normal		10.5	Per cent dry weight
				Range 6.2-20.4	
	10	Hypertension		13.6	
Ewert ¹¹	25	Infections		6.0	Per cent wet weight
	5	Normal		4.99	
	12	Afebrile conditions (including cardiovascular)		2.89	
	17	Febrile conditions (chiefly infections)		0.82	
Woodhouse ⁹	38	Psychiatric disorders with miscellaneous somatic diseases	47.2		Per cent dry weight
Materna and Januschke ^{8b}	12	Normal and cardiovascular disease	16.8		Per cent wet weight
	20	Miscellaneous	5.4		
	8	Infections	2.8		
Chauffard, A Laroche, G, Grigaut, A Ann de med S 149, 1920	3	Normal		5.6	Per cent wet weight
				5.4	
				4.2	
Tex ^{6a}	16	Miscellaneous		9.5	Per cent dry weight
				Range 0.5 to 20.2	
Chauffard, Laroche and Grigaut ^{8b}	32	Miscellaneous		2.8	Per cent wet weight
				Range 0.4 to 8.2	

data do not permit pooling of recorded analyses for purposes of generalization. Table 1 summarizes the mean and extreme values of total lipid and/or cholesterol recorded in the reports of comprehensive quantitative investigations which we have encountered in the literature.

Analyses of the adrenal glands of animals for lipid have generally revealed that lipid, particularly cholesterol, is depleted in infections and severe intoxications, as well as after trauma, hemorrhage, anoxia and violent exercise ^{2c}. Experimental conditions leading to excessive accumulation of adrenal lipid have been studied with much less com-

pleteness and can be briefly summarized. Diets rich in cholesterol have been repeatedly shown to produce accumulation of lipid.^{8b} Splenectomy is reported to result in increased concentrations of adrenal lipid, the mean difference between the control and the splenectomized group, however, is not striking.¹⁶ Excessive storage of cholesterol has been observed in the adrenal glands of rats following prolonged administration of a purified preparation of the adrenocorticotrophic factor of the pituitary gland of the hog,^{2b} although, as is now well known, the uniform effect of acute administration is to produce prominent depletion of both adrenal cholesterol and ascorbic acid, a phenomenon thought to be associated with the elaboration and secretion of adrenal corticosteroids.^{2a} Finally, in rats augmentation of adrenal lipid has been found to follow bilateral nephrectomy,¹⁷ an observation which invites comparison with the increase of serum and liver lipids observed in dogs after bilateral renal ablation or ureteral ligation.¹⁸

Studies of adrenal lipids in experimental hypertension and experimentally produced renal disease remain to be made.

In evaluating the significance of the quantitative alterations which occur in human adrenal lipids in the course of various diseases and disorders, it must be recalled that only a few scattered data are available which describe the normal values. For this reason, comparisons made between the relatively high values observed in hypertension, arteriosclerosis and renal disease, on the one hand, and the low values in acute infections and intoxications, on the other hand, lose much of their validity, since the values cannot be compared with adequate normal standards. Thus, the claim that adrenal lipids are actually higher than normal in the first group of diseases has in most instances been based only on comparisons made with a miscellaneous collection of autopsy material.¹⁹ It might be suspected that such "control" adrenal glands are themselves abnormally low in lipid and that different conclusions might be drawn from the use of more adequately selected normal glands. Indeed, such information as does exist concerning the normal values for human adrenal lipid and cholesterol suggests strongly that while infectious diseases may be associated with striking depletion of adrenal lipid, hypertension and allied disorders do not actually result in elevation of adrenal lipids.²⁰

16 Parhon, C. I., Blinov, A., and Cahane, M. *Compt. rend. Soc. de biol.* **109** 239, 1932.

17 MacKay, E. M., and MacKay, L. L. *J. Exper. Med.* **46** 429, 1927.

18 Winkler, A. W., Durlacher, S. H., Hoff, H. E., and Man, E. B. *J. Exper. Med.* **77** 473, 1943.

19 Borberg^{3a}, Weltmann^{3d}, Knack^{4a}, Chauffard⁵, Felix^{6a}, Landau and McNee^{6b}, Wacker and Hueck^{6c}, Kutschera-Aichbergen^{8a}, Fisher and Hower¹³.

20 Weltmann^{3d}, Felix^{6a}, Landau and McNee^{6b}, Liebegott^{10b}.

The present study deals with a series of 77 pairs of human adrenal glands which were obtained at autopsies and analyzed for the major lipid fractions

MATERIAL AND METHODS

One or both adrenal glands were removed soon after the beginning of the autopsy—usually within two hours. They were kept in a freezing compartment at -18°C until analyzed—generally within two weeks. After being thawed at room temperature, the glands, dissected free of fat and adherent connective tissue, were weighed on a torsion balance to the nearest 0.01 Gm. Both glands were used for the analysis in the majority of cases, portions of each being taken for the determination of both water content and lipid fractions. No attempt was made to separate medulla and cortex, so all analyses refer to whole glands.

Dry weight was determined by weighing an aliquot of 2 to 5 Gm of random fragments from both glands before and after constant weight had been attained in an oven at 70°C . The expression of lipid fractions as percentages of the dry weight of the adrenal glands avoided any influence which variable postmortem dehydration might have on lipid concentration.

The sample for analysis, usually 1 to 3 Gm, after being weighed, was ground to a pulp in a mortar and transferred quantitatively to a flask containing at least 100 cc of a 3:1 mixture of redistilled 95 per cent alcohol and redistilled peroxide-free ether, in which it was refluxed at 70°C for one hour. This extract was then filtered through fat-free paper into a 250 cc volumetric flask, and the sediment of extracted adrenal pulp was washed seven times with 10 to 20 cc portions of the alcohol-ether mixture. The filtrate was made to volume, and aliquots were taken for determination of the various lipid fractions.

Total lipid was determined by weighing the dried residue of a 50 cc aliquot of the original extract evaporated in a water bath at 80°C .

Lipid phosphorus was determined by the method of Fiske and Subbarow²¹ in a 2 cc aliquot of the original extract, essentially as described by Man and Peters²² for blood serum phospholipid.

Fatty acids were determined in a 25 cc aliquot as milliequivalents of titratable acid, after saponification with potassium hydroxide, precipitation by standing, filtration through a Gooch crucible, solution of the residue in hot ethanol and titration with two hundredth normal sodium hydroxide, a method similar to that of Stoddard and Drury²³ as modified by Man and Gildea²⁴.

Cholesterol, both free and total, was determined, after its separation by digitonin precipitation, by measurement of the color developed with sulfuric acid. Details of the method were similar to those described by Bogdanovitch and Man²⁵ except that colorimetric measurement²⁶ was utilized instead of gravimetric determination. Free cholesterol was determined in an aliquot of the original lipid extract, total cholesterol was determined in the aliquot used for the measurement of fatty acids, after saponification, separation and solution in ethanol.

All analyses were made on duplicate aliquots taken from the original alcohol-ether extract.

21 Fiske, C. H., and Subbarow, Y. *J. Biol. Chem.* **66**, 375, 1925.

22 Man, E. B., and Peters, J. P. *J. Biol. Chem.* **101**, 685, 1933.

23 Stoddard, J. L., and Drury, P. E. *J. Biol. Chem.* **84**, 741, 1929.

24 Man, E. B., and Gildea, E. F. *J. Biol. Chem.* **99**, 43, 1932.

25 Bogdanovitch, S. B., and Man, E. B. *Am. J. Physiol.* **122**, 73, 1938.

26 Schoenheimer, R., and Sperry, W. M. *J. Biol. Chem.* **106**, 745, 1934.

The method of extraction used in the present analyses may be subject to criticism because of its relative gentleness as contrasted with long-continued Soxhlet or other continuous extraction methods often employed in the determination of tissue lipids. In a number of instances the completeness of the extraction was checked by subjecting the extracted residue remaining on the filter paper to further extraction by a Soxhlet method involving hot alcohol extraction for twenty-four hours and ether extraction for a subsequent eight hours²⁷. Except in 1 instance, no further significant quantities of lipid were obtained by this more vigorous method. In the exception an additional quantity of lipid amounting to 30 per cent of the lipid originally extracted was recovered in the Soxhlet extract, in this instance, however, an unusually large aliquot of tissue (over 5 Gm) had been inadvertently used. It was noted that the residue of extracted adrenal pulp appeared grossly fatty and gummy on the filter paper and delayed filtration of the alcohol-ether extract for many hours, a phenomenon never again encountered. It was felt that under the conditions described the simple extraction method utilized recovered at least 95 per cent of the total adrenal lipid in all other instances.

A similar comment relates to the method of determining total lipid in the original alcohol-ether extract. In 5 instances, duplicate determinations were made, using the more detailed technic recommended by Bloor²⁸ of rectifying by redissolving in petroleum ether, separating and reevaporating, prior to weighing. In each case the results agreed within 5 per cent with those obtained by the simpler method, and the rectification technic was therefore not routinely used.

Since the rate at which the quantity of any lipid component may change on storage at room and reduced temperatures was of evident interest in connection with the analyses performed, an attempt was made to assess this factor. Adrenal glands obtained at autopsies of dogs were finely minced, and an aliquot taken for immediate analysis. The remaining minced tissue was reserved for later analysis, a part being permitted to remain at room temperature and a part being kept in a freezing locker at -18°C . The results of such comparative analyses are shown in table 2, in which the values of the various lipid components are expressed as percentages of dry weight. The analytic methods used were identical with those used for the human adrenal glands. Agreement was found to be fairly good, with one prominent exception in the unaccountable discrepancies for dog W152. It is of interest that, among the various components, phospholipid and free cholesterol showed the greatest fluctuations and fatty acids were the most stable under these conditions. It may be noted that keeping minced adrenal tissue at room temperature under nonsterile conditions is perhaps an unreasonably strict test for stability. Similarly, mincing alone may favor the loss of lipid in tissue juice, which is not homogeneously represented even in the frozen samples. If consistent and significant changes did occur under the conditions of storage, however, or in the interval between death and autopsy, they were, it is reasonable to surmise, random ones, in the series of analyses reported, and without influence on comparisons made between groups of results in different clinical categories.

In 64 of the 77 autopsies furnishing adrenal glands for analysis the time of autopsy was accurately recorded, and the average period elapsing between death

²⁷ These analyses were made by Dr. Clara M. Szego, of the department of physiological chemistry of Yale University.

²⁸ Bloor, W. R. *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*, New York, Reinhold Publishing Corp., 1943.

and autopsy was approximately seven hours, with a range of from one to eighteen hours. In almost every case, the body was placed in a refrigerated room within two hours after death and kept there until the time of the autopsy.

Calculation of Results—The basic data derived by chemical analysis were values for total lipid, total titratable fatty acids in milliequivalents, lipid phosphorus, free and total cholesterol. By appropriate calculations, values were derived in grams for fatty acids, phospholipid, ester cholesterol and the fatty acid constituents associated with ester cholesterol and phospholipid, respectively. Neutral fat fatty acid was estimated by subtracting phospholipid and cholesterol fatty acid from total fatty acid. Certain assumptions were made in these calculations. A C_{20} fully saturated fatty acid was taken arbitrarily as the average molecular size and the number of grams of fatty acid calculated from milliequivalents of acid on this basis. Lecithin was considered the model phospholipid in calculating grams of phospholipid from grams of lipid phosphorus.

When such calculations were made, it was possible to determine the total lipid in two independent ways: (1) by using the data derived from actual analysis

TABLE 2—*Stability of Lipid Fractions in Adrenal Glands of Dogs Kept at Room and Refrigerator Temperatures**

Dog	Condition	Total Lipid	Fatty Acid	Phospho lipid	Free Choles terol	Ester Choles terol
W 1	At autopsy	60.2	28.5	17.6	6.3	21.1
	Room temperature 72 hr	62.2	28.9	19.8	7.8	20.0
	Freezer 1 week	63.6	31.8	20.3	8.9	21.2
B 53	At autopsy	59.2	29.9	22.9	8.0	19.0
	Room temperature 72 hr	63.6	31.5	18.5	6.5	19.4
	Freezer 72 hr	69.3	30.4	21.1	6.2	20.7
W 323	At autopsy	63.3	33.1	18.7	6.1	20.6
	Room temperature 24 hr	65.9	32.4	13.8	4.5	18.8
	Freezer 1 week	63.0	30.1	16.1	5.6	15.8
W 152	At autopsy	88.5	46.0	31.2	8.5	15.9
	Room temperature 24 hr	63.2	29.3	17.0	3.2	19.9
	Freezer 1 week	62.9	33.9	17.1	4.3	13.0

* Each value is expressed as percentage of the dry weight of adrenal tissue.

of the glands for total lipid and (2) by adding the various independently determined fractions, after making necessary corrections, such as the subtraction from each value for phospholipid of the proportionate weight of the two fatty-acid molecules present in each molecule of lecithin. It is of interest to note the reasonably good agreement between calculated and determined values of total lipid. The average percentage discrepancy in absolute terms between these two values was 8.3, with a standard deviation of 7.6 for a group of 69 sets of adrenal glands.

Clinical Categories—Study of the clinical and autopsy records of persons whose adrenal glands were subjected to lipid analysis permitted the construction of five different categories: 1. Normal persons, dead as the result of violence, without evidence of significant disease. 2. Miscellaneous patients, whose deaths were due to a variety of diseases commonly encountered in a general hospital but not to significant infection, hypertension or any marked grade of arteriosclerosis. 3. Persons whose deaths resulted from infections, chiefly severe septic infections such as peritonitis, septicemia, meningitis and bacterial pneumonias. 4. Persons who died with arteriosclerosis, i.e., with marked atherosclerosis involving major vessels, usually those of the heart, the brain and the kidneys. Most deaths in this group were considered the result of coronary insufficiency, with or without

myocardial infarction Hypertension could be excluded on the basis of adequate clinical data covering months or years prior to death, absence of significant cardiac hypertrophy and absence of renal arteriosclerosis 5 Persons who died with long-sustained hypertension, usually at high levels, and associated with marked cardiac hypertrophy and arteriosclerosis of the kidney and other organs All the cases selected met the two criteria suggested by Moritz and Oldt²⁹ sustained blood pressure levels greater than 150 systolic and 100 diastolic or 160 systolic and 90 diastolic and a heart weight greater than 400 Gm in males or 350 Gm in females

RESULTS

In table 3 are shown the data concerning the lipid components derived from analyses of 77 pairs of adrenal glands under the clinical groupings cited in the previous section In table 4 these data are summarized in the presentation of the mean values and mean standard errors for total lipid, fatty acid, phospholipid, free and ester cholesterol, each expressed both as percentage of the dry weight of the glands, as total quantity in both adrenal glands and as percentage of total lipid Confidence limits for significant differences are taken as a p value less than 0.01

Significant differences of the mean values for the dry weight of both adrenal glands were not detected between any two of the clinical groups Borderline significance (p less than 0.02) may be attributed to the difference between 2.9 Gm for the mean adrenal weight in the hypertensive group and 2.1 Gm for that in the infectious group

The value of total lipid expressed as percentage of dry weight of adrenal tissue was not significantly different in the hypertensive, the arteriosclerotic and the normal group In each of these groups, however, there was a significantly greater concentration of lipid in the adrenal glands than in the infectious disease group Adrenal glands of the hypertensive and the normal group also contained significantly more total lipid than those of the miscellaneous group In the arteriosclerotic group this value was greater than in the miscellaneous group by an amount of borderline significance (p less than 0.02) The infectious and miscellaneous groups did not differ significantly in their adrenal lipid levels

Similar relationships are seen on comparing the total quantities of adrenal lipid present in the various groups, with the exception that the small number of values for the normal group (owing to the difficulty of obtaining both entire adrenal glands at the autopsies of normal persons) makes statistical comparison invalid apart from enormous differences

In terms of percentage of dry weight of adrenal tissue, roughly the same relations hold for fatty acid as for total lipid among the various groups Adrenal glands of normal, hypertensive and nonhypertensive arteriosclerotic persons were not statistically distinguishable, while those of the miscellaneous group contained a greater concentration of fatty acids than those of the infectious group by a factor of only borderline significance Greater concentrations of fatty acid were found in the normal, hypertensive and arteriosclerotic groups than in the infectious group

Examination of the values for total quantity of fatty acid indicates similar relationships, with the exception again that the small number of normal values eliminates this category from statistical consideration because of the high standard error associated with even slight variation in a small population When fatty acid was considered as percentage of total lipid, an entirely different set of rela-

29 Moritz, A. R., and Oldt, M. R. *Am J Path* 13: 679, 1937

TABLE 3—Weights and Lipid Fractions of Human Adrenal Glands*

Sex	Age	Principal Clinical and Autopsy Observations	Wet Wt Both Adrenal Glands, Gm	Dry Wt Ratio	Dry Wt Both Adrenal Glands, Gm	Gm per 100 Gm Dry Weight				
			Normal Group			Total Lipid	Fatty Acid	Phos pholipid	Free Choles terol	Ester Choles terol
I	34	Death in automobile accident—transection of spinal cord	4.4	0.41	1.3	63.1	36.3	9.9	7.2	24.5
M	30	Instantaneous death from gunshot wound of chest	5.8	0.46	2.1	62.5	29.1	9.4	6.1	30.0
M	45	Instantaneous death from injury of the head		0.41		65.4	36.8	8.3	3.0	32.7
M	51	Instantaneous death from injury of the head	7.6	0.31	2.4	53.3	47.7	9.4	3.8	21.9
M	60	Death from fall	12.9	0.42	5.4	64.3	28.3	5.2	9.2	22.4
M	50	Stab wound through heart		0.37		68.0	33.8	10.4	6.3	31.8
I	24	Found dead with pulmonary embolus, 3 months pregnant		0.33		58.8	26.2	11.4	6.9	26.4
I	8	Instantaneous death in automobile accident—fractured skull		0.38		59.7	29.4	16.6	6.3	18.0
M	26	Fractured skull, epilepsy		0.24		48.9	24.1	12.7	7.7	14.6
M	35	Found dead after fight—probable strangulation		0.32		58.4	30.9	11.2	9.0	15.9
M	24	Died shortly after stab wound of heart		0.43		63.9	32.1	8.1	6.2	22.7
M	34	Suicide with carbon monoxide	6.0	0.39	2.3	55.8	26.6	8.1	7.9	20.5
M	21	Instantaneous death from bullet wound of chest		0.28		62.8	32.0	8.6	6.2	22.7
Hypertension Group										
M	54	Severe hypertension 10 years, death during sympathectomy		0.45		59.9	38.9	12.2	1.4	21.1
M	47	Severe hypertension 8 years, terminal renal and cardiac failure	10.3	0.26	2.7	53.0	31.8	14.8	2.5	12.2
I	67	Hypertension 11 years, terminal cardiac failure	10.1	0.35	3.5	63.0	30.4	8.8	1.2	26.2
I	37	Hypertension 3 years, severe diabetes and glomerulosclerosis	7.9	0.30	2.4	42.6	22.4	10.4	2.4	28.0
F	66	Hypertension 2 years, diabetes and chronic pyelonephritis	7.4	0.33	2.4	53.8		8.8	2.7	22.4
I	48	Hypertension, cardiac failure 1 year, terminal uremia	9.9	0.28	2.8	61.1	30.7	10.4	3.0	21
Arteriosclerosis Group										
I	65	Diabetes, myocardial infarct	3.9	0.55	1.4	55.0	35.2	12.0	1.3	15.6
F	54	Myocardial infarct, terminal uremia	6.6	0.32	2.1	57.2	30.9	11.7	3.9	13.2
M	77	Acute congestive failure, large myocardial scars	7.4	0.37	2.7	65.5	31.8	7.5	6.1	26.3
M	57	Myocardial infarct	6.2	0.40	2.5	64.6	33.8	13.3	3.6	21.7
I	60	Coronary occlusion without infarct, mild diabetes	5.2	0.33	1.7	52.0	26.0	12.7	4.5	17.1
M	56	Coronary occlusion without infarct	6.1	0.37	2.1	61.8	28.2	8.8	7.6	24.3
Miscellaneous Group										
I	36	Glioblastoma cerebri, death 1 day after craniotomy	7.8	0.35	2.7	51.0	25.6	12.2	2.3	17.0
M	59	Chronic prostatitis, cystitis, pyelonephritis, uremia	7.4	0.29	2.9	65.2		7.5	1.1	19.3
I	15	Acute polioencephalomyelitis, death from respiratory paralysis	7.7	0.26	2.0	34.4	20.7	15.6	1.9	9.2
F	27	Death in diabetic coma	8.7	0.28	2.4	36.4	21.3	14.6	2.8	15.6
M	41	Portal cirrhosis	5.5	0.20	1.1	51.7	32.4	15.6	3.7	11.2
M	54	Syphilitic aortitis, stenosis of coronary ostia, cardiac failure	5.8	0.31	1.8	47.3	26.3	11.7	4.3	10.2
Infection Group										
M	77	Pneumococcal lobar pneumonia		0.34		43.0	26.1	8.3	1.6	5.0
F	55	Empyema of gallbladder with peritonitis	8.4	0.25	2.1	47.9	26.6	13.0	1.6	9.5
F	80	Lobar pneumonia	5.7	0.27	1.5	32.8	23.6	14.0	1.0	11.4
F	33	Perforation of uterus and peritonitis post partum	8.4	0.32	2.7	24.7			11.9	0.8
M	57	Acute pyelonephritis, hypertension, uremia	10.9	0.25	2.7	41.5	18.7	14.0	4.6	9.2
M	3	Meningococcemia, Waterhouse-Friderichsen syndrome	5.8	0.32	1.9	23.2	15.6	8.1	5.9	5.1

* Values for all the members of the normal group and for 6 representative persons in each of the other groups are shown. The total number of persons whose adrenal lipid values were determined included 19 with hypertension, 15 with arteriosclerosis without hypertension, 18 with miscellaneous disorders and 12 with septic infections.

tionships appeared. Now the highest values were found in the groups of persons dying of infectious and miscellaneous diseases—statistically indistinguishable from each other—and both significantly different from the remaining three groups.

With phospholipid expressed as percentage of dry weight of adrenal tissue, the notable feature of a comparison of any two of the five groups was the relative constancy of the values for phospholipid, there being no significant differences between any of the groups. Not only mean values but individual values as well (see table 3) showed relatively great consistency as compared with the other fractions measured.

Expressed as percentage of total lipid, phospholipid followed the same pattern in the various groups as did fatty acid, being greatest in the miscellaneous and infectious disease group and smallest in the arteriosclerotic, hypertensive and normal categories.

TABLE 4—Mean Values of Adrenal Lipid Fractions in Various Clinical Categories*

	Normal	Hypertension	Infection	Arteriosclerosis	Miscellaneous
Dry weight of adrenal tissue	2.8 ± 0.66 (5)	2.9 ± 0.23 (18)	2.1 ± 0.18 (11)	2.6 ± 0.24 (14)	2.3 ± 0.19 (14)
Total lipid, % dry weight	60.3 ± 1.94 (13)	57.7 ± 1.51 (19)	37.0 ± 3.32 (11)	55.9 ± 2.44 (15)	45.9 ± 3.13 (17)
Total lipid, total amount	1.7 ± 0.79 (5)	1.7 ± 0.14 (18)	0.8 ± 0.10 (10)	1.5 ± 0.09 (14)	1.1 ± 0.12 (14)
Fatty acid, % dry weight	30.1 ± 1.12 (13)	29.0 ± 0.09 (18)	21.4 ± 1.28 (11)	29.9 ± 1.09 (15)	26.4 ± 1.45 (16)
Fatty acid, total amount	0.8 ± 0.17 (5)	0.9 ± 0.07 (17)	0.4 ± 0.04 (10)	0.8 ± 0.08 (14)	0.6 ± 0.05 (13)
Fatty acid, % total lipid	49.7 ± 1.12 (13)	50.4 ± 1.31 (18)	56.9 ± 2.83 (10)	54.0 ± 1.72 (15)	58.4 ± 2.48 (15)
Phospholipid, % dry weight	9.9 ± 0.76 (13)	10.2 ± 0.57 (19)	11.9 ± 0.87 (10)	11.0 ± 0.60 (14)	12.6 ± 1.90 (15)
Phospholipid, total amount	0.21 ± 0.017 (5)	0.28 ± 0.016 (18)	0.29 ± 0.036 (9)	0.26 ± 0.020 (13)	0.28 ± 0.022 (14)
Phospholipid, % total lipid	16.7 ± 1.5 (13)	18.1 ± 1.35 (19)	33.0 ± 4.0 (9)	20.9 ± 2.0 (14)	28.0 ± 2.9 (15)
Free cholesterol, % dry weight	6.6 ± 0.49 (13)	4.9 ± 0.59 (19)	3.4 ± 0.94 (12)	3.4 ± 0.47 (15)	3.3 ± 0.58 (17)
Free cholesterol, total amount	0.20 ± 0.078 (5)	0.15 ± 0.022 (18)	0.08 ± 0.026 (11)	0.09 ± 0.018 (14)	0.07 ± 0.019 (14)
Free cholesterol, % total lipid	11.2 ± 0.89 (13)	8.5 ± 0.95 (19)	11.1 ± 4.2 (11)	5.9 ± 0.7 (15)	7.1 ± 1.19 (16)
Ester cholesterol, % dry weight	23.2 ± 1.62 (13)	21.5 ± 1.27 (19)	7.9 ± 2.04 (12)	20.5 ± 2.06 (15)	12.9 ± 1.86 (17)
Ester cholesterol, total amount	0.70 ± 0.14 (5)	0.65 ± 0.063 (18)	0.32 ± 0.097 (11)	0.57 ± 0.056 (14)	0.36 ± 0.20 (14)
Ester cholesterol, % total lipid	38.2 ± 2.14 (13)	37.5 ± 2.35 (19)	18.3 ± 4.09 (11)	36.5 ± 3.88 (15)	28.1 ± 3.75 (16)

* Each of the values shown is the mean and the standard error. The number in parentheses is the number of persons in the group. All values not percentages are in grams.

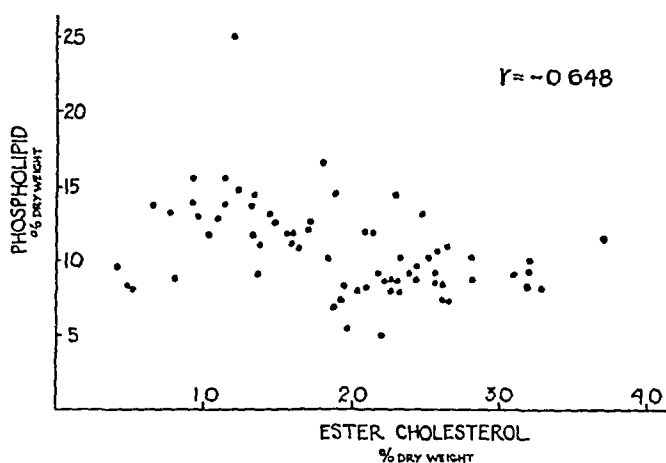
The concentration of free cholesterol per unit of dry weight was found to be highest in the normal group, less high (although not significantly so) in the hypertensive group and relatively consistently low in the remaining three groups. In considering the total amount of free cholesterol present in both adrenal glands it is of interest to note the absence of significant differences except between the hypertensive and the miscellaneous group. Expressed as percentage of total lipid, free cholesterol was highest in the normal group, lowest in the arteriosclerotic group—between which two values the only difference of significance was detected.

Ester cholesterol expressed as percentage of dry weight of adrenal tissue showed considerable variation among the individuals of certain groups, notably the infectious disease and miscellaneous groups, as may be observed by inspecting the relative magnitude of the standard error of the mean. Despite large intragroup variations, however, the difference between certain of the group means was so great as to permit an appearance of significance. As with total lipid, the five clinical categories fell into two larger groups, one of relatively high ester cholesterol content, comprising arteriosclerotic, hypertensive and normal persons, the other with low ester cholesterol content comprising the persons whose deaths were ascribed to

miscellaneous diseases or to infectious diseases but who were neither hypertensive nor arteriosclerotic. Significant differences did not appear between the individual categories within each of these two larger groups.

Values for the total quantity of ester cholesterol in both adrenal glands were statistically distinguishable only on comparing the hypertensive and the infectious disease group. Expressed as percentage of total lipid, however, ester cholesterol was significantly higher both in normal and in hypertensive persons than in persons in the infectious or miscellaneous disease groups.

Relatively consistent values, both for individuals and for the clinical groups, were observed for two constituents: fatty acids, expressed as percentage of dry weight of adrenal tissue (range of variation from 21.4 for the infectious disease group to 30.1 for the normal group) and expressed as percentage of total lipid (range of variation from 49.7 for the normal group to 58.4 for the miscellaneous disease group), and phospholipid, expressed as percentage of dry weight of adrenal tissue (ranging from 9.9 for the normal group to 12.6 for the miscellaneous disease group) and expressed as total quantity (ranging from 0.21 Gm for the



Relation between the phospholipid and ester cholesterol concentrations in human adrenal glands

normal group to 0.29 Gm for the infectious disease group). Not only the limited range of mean values but also the relatively small standard errors distinguished these components in their consistent levels.

An additional point of interest is suggested by the reciprocal relationship between phospholipid and ester cholesterol manifest in table 4. The groups with the highest ester cholesterol values (normal, hypertensive and arteriosclerotic persons) had, associated with these values, the lowest phospholipid values, whether these were expressed as percentage of dry weight of adrenal tissue, as total quantity present or as percentage of total lipid. Further investigation of this relationship revealed that a significant inverse correlation existed between ester cholesterol and phospholipid (both expressed as percentage of dry weight) when the two values were compared in each pair of adrenal glands analyzed. The correlation coefficient, r , was calculated to be -0.648 , and was associated with a p value much less than 0.01 (figure).

COMMENT

The results reported in this study indicate that several lipid components of adrenal glands (total lipid, ester cholesterol, fatty acids)

are not present in concentrations higher than normal in persons with significant hypertension or arteriosclerosis, a finding which may also be inferred from previously reported data. It seems equally well established that a group of patients with atherosclerosis but free of hypertension would be virtually indistinguishable from a group of hypertensive persons with respect to adrenal lipids. There is a greater or less degree of depletion of these lipids in the adrenal glands of persons dying with a variety of other diseases, most notably infections.

The impression of many previous investigators that supernormal lipid levels are found in the adrenal glands of hypertensive patients seems to have been based on the unjustified use of a group of non-hypertensive persons with miscellaneous diseases as a reference group with which to compare the hypertensive group in judging adrenal lipid values. The data presented in the present report support the conclusion that adrenal lipids are not abnormally elevated in instances of hypertensive and arteriosclerotic disease, but remain at normal levels not maintained in other disorders.

A purely descriptive correlative study like the present one involves certain inherent difficulties of interpretation, further complicated by the use of discontinuous categories in which to classify a continuum of data. First, it may be recognized that clinical groupings of the sort made are meaningful for nosologic purposes but may actually conceal significant physiologic factors causally associated with changes in tissue lipids. Thus, the factors determining the levels of adrenal lipid fractions may be unconsciously selected correlates of the clinical groups chosen.

In a descriptive study, hundreds of possible correlates of the classifications made suggest themselves, and can be eliminated only by a number of analyses sufficient to permit isolated statistical evaluation of every conceivable contributory factor. A few such possibilities are: 1. Presence of associated metabolic disturbances, such as diabetes, which occurred in a considerable number of the hypertensive group. 2. Occurrence of renal failure, certainly a much commoner terminal event in hypertensive and arteriosclerotic disease than in the other clinical groups. The observations already referred to, demonstrating an increase in the lipid content of adrenal and other tissues following nephrectomy and ureteral ligation, make this possibility more than a remote one. 3. General nutritional state, which might be more influenced by infection than by cardiovascular or renal disease.

Isolation and separate analysis of these factors are not possible in a study embracing relatively few analyses. A few simple factors, however, were subject to estimation. Sex could be eliminated, since in all but the normal group there was no preponderant sexual selection.

of cases, the numbers of men and women being approximately equal in the hypertensive and the infectious disease group. Age did not seem to be a factor, although the mean for the miscellaneous group was relatively low (37 as contrasted with 51, 63 and 64 for the infectious disease, arteriosclerosis and hypertension groups, respectively), all ages were represented in each group. Duration of illness and hospitalization may be considered a factor of possible importance. If adrenal cholesterol is reduced following stresses of wide variety, duration of disease might play a role in determining the level of adrenal cortical lipid at death. Furthermore, the arteriosclerotic group might be suspected to include a large proportion of deaths following brief and sudden illnesses, since both myocardial infarction and cerebral hemorrhage were common events in this group. Examination of this factor, however, did not reveal any prominent difference between mean numbers of hospital days for any two groups, the values being 9.6, 11.8, 17.5 and 13.7 for the groups of patients with arteriosclerosis, infections, miscellaneous diseases and hypertension, respectively. A considerable range was present in each group.

In any event, lengthy speculation as to the possible biologic or pathologic significance of the results determined seems supererogatory. The depletion of adrenal cholesterol and, as appears from the present study, fatty acids, in infectious diseases has already been clarified by experimental work relating this phenomenon to an increased rate of secretion of corticosteroid substances. The less prominent, but still distinct, reduction of the same lipids in the many other disease states may tentatively be considered a similar response of the pituitary-adrenal cortical system to undefined stimuli incident to a variety of metabolic disturbances.

The unique maintenance of adrenal cortical lipids at normal levels in persons with hypertensive and arteriosclerotic disease offers another facet of similarity in these two groups. Whether a single common factor operative in both groups is responsible, or whether a fortuitously similar state results from independent mechanisms cannot be discerned. Disturbances of cholesterol metabolism, which are of current interest in the experimental investigation of atherosclerosis, may be related to the preservation of relatively high adrenal cholesterol levels in the arteriosclerotic patient. Similarly, proper interpretation of the comparable state of adrenal lipids in the hypertensive person may take its place in the present fragmentary array of evidence relating hypertension to the state of adrenal cortical function.³¹

30 MacKay and MacKay¹⁷ Winkler and others¹⁸

31 Perera, G. A. J. A. M. A. **129** 537, 1945 Shorr, E. Am J Med **5** 783, 1948

A final logical solecism must be admitted. The foregoing discussion is predicated on the assumption that in hypertension and arteriosclerosis, adrenal lipid levels are defended against stimuli common to all disorders, which would otherwise lower them by the time of death. It seems perhaps more justified to conclude that preservation of these levels results simply from absence of adequate stimuli of the sort present in infectious and other nonvascular diseases. Also, since the level of any given tissue component is the resultant of several rates, involving production, utilization and transport, it cannot be said whether preservation of normal levels of lipid in the instances noted may not reflect altered, though balanced, rates of accumulation and removal.

SUMMARY

Analyses of human adrenal glands to determine the levels of the major lipid components were conducted on material derived from 77 autopsies, and the results were compared in several clinical classifications. A group of persons dying as the result of violence furnished material for the description of normal data, represented in the literature poorly or not at all. Certain lipid fractions, notably ester cholesterol, were present in similar concentrations in hypertension and arteriosclerosis, being maintained at levels comparable to those found in adrenal glands of persons dying without disease. In infections and in most disorders other than those characterized by marked hypertension or arteriosclerosis, the concentration and the total quantity of these components were markedly diminished. The quantity of phospholipid, like that of fatty acid, tended to be quite constant both in individuals and in the various clinical groups, for which averaged values were recorded. Phospholipid expressed as a percentage of the dry weight of adrenal tissue varied inversely with total lipid and ester cholesterol, being highest in the infectious disease and miscellaneous disease groups and lowest in the hypertensive and normal groups. This relationship was even clearer when phospholipid was expressed as a percentage of the total lipid present.

ACTION OF IODINE ON GOITERS PREVIOUSLY TREATED WITH THIOURACIL

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THE THERAPEUTIC use of substances, such as thiouracil, which have a powerful functional and morphologic effect on the thyroid gland has introduced a certain confusion into the anatomoclinical interpretation of goiter, which until 1943 (Buño¹) rested on a firm base

Hyperplasia of the thyroid gland (very large in some cases) was found experimentally, which was characterized by histologic signs of hyperactivity and yet was accompanied by hypothyroidism. The mechanism of this apparently paradoxical and contradictory behavior was made clear by further experimental work.

At the Massachusetts General Hospital, under the direction of Means and co-workers² comparisons were made between specimens of the thyroid gland obtained prior to thiouracil treatment and the gland removed after such treatment. Following administration of thiouracil, a slight increase of the average height of cells had been observed, also general proliferation and hyperplasia of the gland with increased vascularization and in some instances hyperplasia of interstitial lymphocytic tissue. In 7 patients treated by them, first with thiouracil alone and then with thiouracil and iodine together, the initial increase in average cell height obtained with the first therapy was followed by a decrease when iodine was given. Although the authors have not given a histologic description of their preparations, their photomicrographs indicate that the iodine administered with the thiouracil not only modified the height of cells but also the amount of stored colloid. The gland showed a medium degree of activity with numerous absorption vacuoles.

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1 Buño W. Estudios de histofisiología e histopatología tiroidea, Buenos Aires, El Ateneo, 1943

2 Rawson, R W, Evans, R D, Means, J H, Peacock, W C, Lerman, J, and Cortell, R E. J Clin Endocrinol 4 1, 1944. Rawson, R W, Moore, F D, Peacock, W C, Means, J H, Cope, O, and Riddell, C B. J Clin Investigation 24 869, 1945

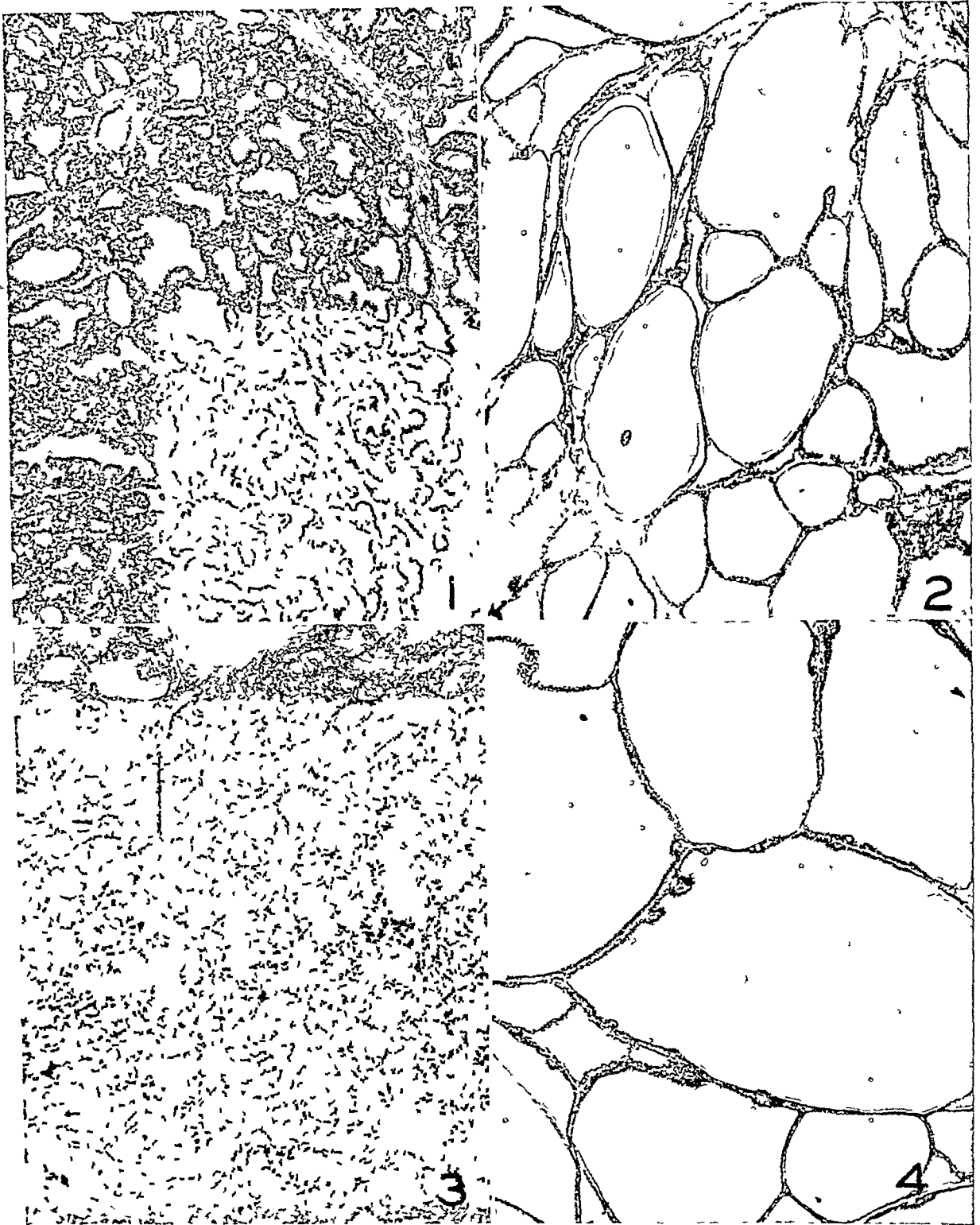


Fig 1—Lobe of the thyroid gland removed after the patient had been treated solely with thiouracil ($\times 420$, basal metabolic rate, + 7)

Fig 2—Second lobe removed from the same patient four months after that in figure 1. The preoperative treatment was solely with iodine ($\times 420$, basal metabolic rate, + 35)

Fig 3—Lobe removed after the patient had been treated solely with thiouracil ($\times 420$, basal metabolic rate, + 15)

Fig 4—Second lobe removed from the same patient as that in figure 3 four months later. The preoperative treatment was solely with iodine ($\times 420$, basal metabolic rate, + 42)

Halpert, Cavanaugh and Keltz³ made a histopathologic study of thyroid glands of patients with exophthalmic goiter, 2 of whom had been treated solely with thiouracil, 3 with iodine followed by thiouracil and 2 with thiouracil first, then with thiouracil and iodine combined. The last two interest us most. One of them had received iodine four months before starting to take thiouracil, and in the course of treatment given before a second operation, was treated with thiouracil and iodine combined, all of which makes the interpretation of the case complicated. The other of these 2 patients was operated on in one stage, so that there is no proof as to what the state of the gland was before the iodine treatment, and, besides, the iodine was given together with thiouracil.

The thyroid glands studied by us, from patients with exophthalmic goiter⁴ who prior to operation had been treated with thiouracil, showed the characteristic appearance of intense hypersecretion, even when the metabolism was normal or below normal and the patient presented signs of hypothyroidism. But we particularly wish to draw attention to 2 cases in which thyroidectomy was carried out in two stages with a four month interval between the operations.

Thiouracil had been given prior to the first operation, and the histologic appearance was that of the hyperplasia and hyperactivity known to be produced by this drug (figs 1 and 3).

On the other hand, prior to the second operation iodine alone was used in both patients, and the histologic picture obtained was remarkably different from that of the lobe removed four months earlier (figs 2 and 4). The thyroid follicles were enlarged, lined with low epithelium, and contained abundant dense colloid, in fact, the picture corresponded to that of colloid goiter. At some points, however, there were still to be seen a few small follicles with epithelium of medium height.

The contrast between these two types of structure can be seen by comparing the corresponding photomicrographs (for all sections the magnification is the same).

COMMENT

The administration of thiouracil produces a marked reduction of the iodine content of the normal thyroid gland (Astwood and Bissell⁵, Franklin, Chaihoff and Lerner⁶). The gland depleted of iodine can again store large quantities of it when iodine is administered intravenously after suspension of thiouracil treatment (Astwood⁷).

3 Halpert, B., Cavanaugh, J. W., and Keltz, B. F. *Arch Path* **41** 155, 1946.

4 The material was obtained from the Institute of Endocrinology. The clinical study was made by Assistant Prof. J. M. Cerviño, the operation was done by Dr. G. Caprio.

5 Astwood, E. B., and Bissell, A. *Endocrinology* **34** 282, 1944.

6 Franklin, A. L., Chaihoff, I. L., and Lerner, S. R. *J Biol Chem* **153** 265, 1944.

7 Astwood, E. B., in *The Harvey Lectures, 1944-1945*, Lancaster, Pa., Science Press Printing Company, 1945, vol. 40, p. 195.

The experiments of VanderLaan and Bissell,⁸ in which iodine and thiouracil were given simultaneously, seem to indicate that the gland can in reality metabolize large quantities of iodine but is totally incapable of storing it. Hence, the thyroid tissue does not contain iodine in any appreciable quantity if this is determined a few hours after administration of the element. On the other hand, thirty minutes after an injection, the content of iodine is considerable.

Mackenzie,⁹ administering combinations of goitrogenic substances with adequate quantities of sodium iodide, was able to prove that iodine completely inhibits the hyperplastic reaction produced in the thyroid gland by thiouracil. On the other hand, it is interesting to note that it increases the hyperplastic reaction produced by sulfaguanidine.

Our observations seem to indicate that likewise in patients with exophthalmic goiter thiouracil increases thyroid avidity for iodine. As has been shown by many observations, iodine alone is not capable of producing in the thyroid gland of a patient with exophthalmic goiter that state of colloid transformation which is reached when iodine is given after the administration of thiouracil. This would agree with experiments of Larson, Keating, Peacock and Rawson,¹⁰ who demonstrated in the chicken that when the administration of thiouracil is stopped the capacity of the thyroid gland to store radioactive iodine increases greatly.

Besides, the histologic appearance contrasts with the clinical picture, for in both cases at the moment of the first stage of operation the basal metabolism was lower than at the second stage, while the histologic appearance seemed to indicate the contrary (basal metabolic rate +7 before and +35 afterward in one case and +15 before and +42 afterward in the other).

SUMMARY

Two thyroid glands have been studied in lobectomy in two stages in patients with exophthalmic goiter. Before the first operation treatment was with thiouracil alone, before the second, four months later, iodine alone was given. Sections from lobes removed at these two operations, when compared, showed a remarkable colloid transformation of the gland.

This seems to justify the conclusion that iodine has a far greater effect on the thyroid gland if the patient has previously been treated with thiouracil and this treatment has been suspended, likewise, that the effect thus obtained is much greater than that obtained exclusively with iodine.

8 VanderLaan, W. P., and Bissell, A. *Endocrinology* **39** 157, 1946.

9 Mackenzie, C. G. *Endocrinology* **40** 137, 1947.

10 Larson, R. A., Keating, F. R., Jr., Peacock, W., and Rawson, R. W.: *Endocrinology* **36** 149, 1945.

DEPOSITION AND FATE OF PLUTONIUM, URANIUM AND THEIR FISSION PRODUCTS INHALED AS AEROSOLS BY RATS AND MAN

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SAN FRANCISCO

THE MANNER in which foreign materials are eliminated from the lungs has long been of interest Robertson¹ has summarized previous investigations In general, it has been shown that particles inhaled into the lungs may be eliminated in two ways In both mechanisms the particles are engulfed by phagocytes Following this, some particles, such as silica, are carried into the lymphatic system of the lung by phagocytes migrating through the alveolar lining² Concerning the other mechanism of elimination it is postulated that particles are transported as far as the ciliated epithelium of the respiratory bronchioles, where they are swept out by ciliary action The cells responsible for transporting them to the respiratory bronchioles have been identified as dust or septal cells Fried³ expressed the belief that they are mesenchymal in origin

McCutcheon's⁴ studies of chemotaxis have shown that the phagocytes of the lung are specialized with respect to the kind of particle which they will engulf He listed polymorphonuclear cells as being positively chemotactic to collodion particles, bacteria, and tissue proteins, negatively chemotactic to aluminum silicate and indifferent to carbon particles In this respect they are in contrast to dust or septal cells, which phagocytose carbon Dust cells and monocytes do not display positive chemotaxis but accomplish their work by means of random locomotion

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From the Divisions of Radiology and Medicine, University of California Medical School, San Francisco, and the Division of Medical Physics and Crocker Laboratory, University of California, Berkeley, Calif

1 Robertson, O H *Physiol Rev* **21** 112, 1941

2 Cole, L G, and Cole, W G *Pneumoconiosis (Silicosis) The Story of Dusty Lungs*, a Preliminary Report, New York, John B Pierce Foundation, 1940

3 Fried, R M *Arch Path* **17** 76, 1934

4 McCutcheon, M *Arch Path* **34** 167, 1942

The hazards of various industrial materials in the lungs have been of concern for many years. Pulmonary elimination and/or absorption of materials became a matter of even greater concern with the release of nuclear energy and the attendant possibility of the accidental release of the products of nuclear fission. However, the production of fission products and fissionable materials had its compensations as far as radiation hazards were concerned, in that the materials which were possible sources of danger because of their radioactivity were by the same token useful tools in the study of the function of the lungs.

Of the several technics available for studying the transport of radioactive materials in the body two were used in these studies. One was the quantitative determination of the behavior of the radioactive materials administered by observing the fate of a known amount of active material introduced into the lungs. This was done by measuring the radioactivity of the animal tissues and excreta with a Geiger-Muller counter in the detection of beta and gamma radiation and the parallel plate linear amplifier combination in that of alpha particles. The second was the determination of the exact location of the particles in lung tissue. This can be done through the use of the technic of radioautography. Radioautographs were taken of lung sections at several periods of time after the lungs had been exposed to the radioactive aerosols used in these studies.

The information gained from these two methods places the investigation on a quantitative basis. Since the materials can always be followed because of their radioactivity, it is possible to determine the various fractions following any particular route as well as the time it takes for translocation to occur.

The elements used were administered as smokes and sprays. The smokes and sprays were composed of particles of plutonium, uranium and the fission products of both of these materials. The mixture of radioactive fission products contained principally strontium (Sr), yttrium (Y), zirconium (Zr), columbium (Cb), ruthenium (Ru), barium (Ba), lanthanum (La), cerium (Ce), cesium (Cs), praseodymium (Pr), neodymium (Nd) and element 61 at the time of the assay of radioactivity in organs, liver, and excreta. Zirconium and protoactinium (Pa) were studied separately as well.

METHODS OF STUDY

The manner in which radioactive material is assayed when present in biologic tissues has been adequately described elsewhere⁵

5 (a) Hamilton, J. G. The Metabolism of Carrier-Free Fission Products in the Rat, MDDC 1275, June 1946. Technical Information Division, U. S. Atomic Energy Commission, Oak Ridge, Tennessee. (b) Scott, K. G., Axelrod, D. J., Crowley, J., Lanz, H., and Hamilton, J. G. Studies on the Inhalation of Fissionable Materials and Fission Products and Their Subsequent Fate in Rats and Man, MDDC 1276, October 1946. (c) Hamilton, J. G. Radiology 49: 325 and 343, 1947.

The methods which were used to deposit radioactive aerosols in the lungs of rats are as follows. With the exception of the one study of a human subject, young rats weighing between 200 and 300 Gm were used in all experiments. The rats were prepared for exposure with an intraperitoneal injection of pentobarbital sodium. The dose was 40 mg per kilogram of body weight for male rats and 30 mg per kilogram for female rats. After the rat was anesthetized, the whiskers on the nose were closely clipped. The lips were fastened together with a three-cornered stitch between the lower lip and the two halves of the upper lip. The mouth was further sealed up with celloidin (a concentrated pyroxylin), and at the same time the glass nose piece was cemented in place (fig 1). This procedure completely sealed off the openings of the mouth and directed the rat's respiration through the nasopharynx. In order to complete the exposure, the glass nose piece of the rat was plugged into the aerosol chamber with gum rubber tubing (figs 2

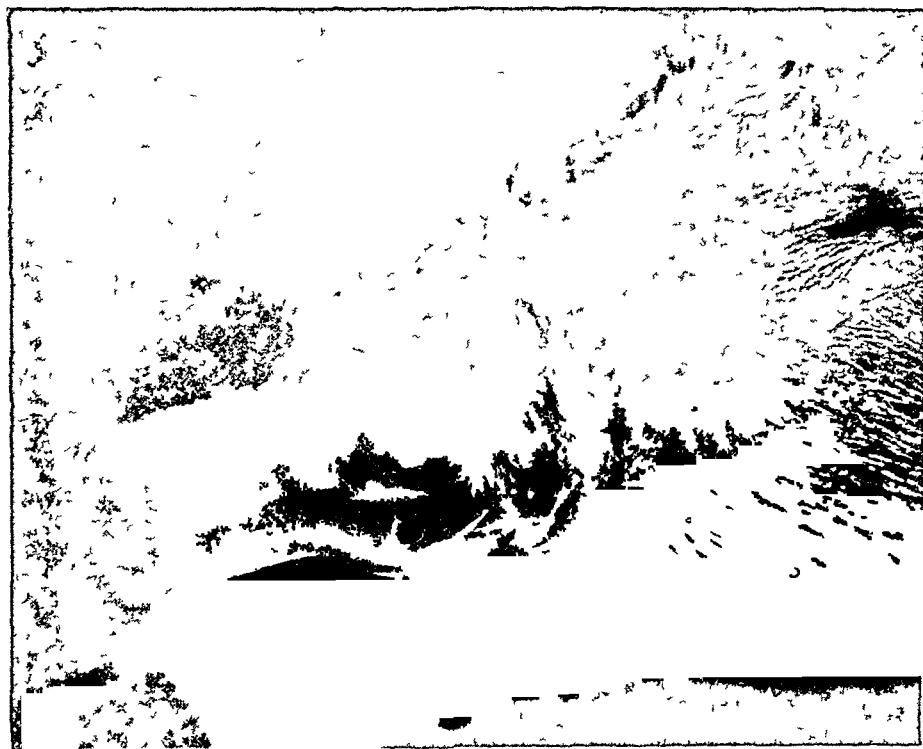


Fig 1—A rat prepared for exposure to aerosol, with nose piece in place and mouth sealed with celloidin

and 3). After being exposed to the aerosol under study, the rats were placed in metabolism cages so that fecal and urinary excretions could be collected separately. The animals were killed at varying times after exposure, and their tissues, urine and feces were assayed for the element being studied.

The animals used in these studies were given the following materials as aerosols:

- 1 Plutonium oxide (PuO_2) produced by burning both the metal and the salts of plutonium
- 2 Plutonium nitrate ($\text{PuO}_2(\text{NO}_3)_2$) as a spray
- 3 Fission products combined with their parental elements, plutonium and uranium, which had been previously exposed to intense and prolonged neutron

irradiation Both the plutonium and the uranium were burned in the metallic state, thus producing aerosols of their oxides and the oxides of most of the fission products

4 Carrier-free fission products, free of uranium and plutonium and containing very small amounts of inactive isotopic elements They are described as being carrier free because of the fact that they were prepared without inclusion of their stable isotopes during their chemical separation from uranium and plutonium Most of these also were primarily in the form of their oxides

5 Aerosols of carrier-free zirconium or zirconium oxide (ZrO_2)

6 Aerosols of protoactinium as protoactinium oxide (Pa_2O_6)

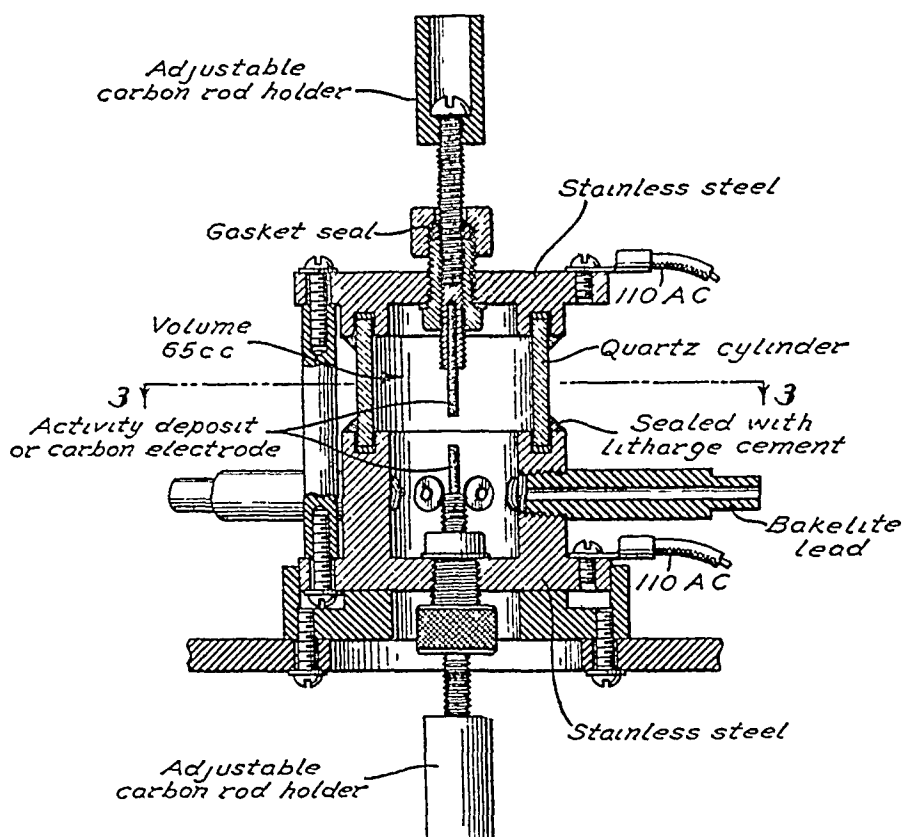


Fig 2—The exposure chamber used to produce aerosols by burning the radioelement, deposited on graphite electrodes, in an electric arc This was done in an atmosphere of pure oxygen in order to prevent the formation of poisonous amounts of carbon monoxide The bakelite leads were connected to the rat's nose piece with gum rubber tubing The exposure was made by striking an electric arc between the two electrodes The exposure time was about 30 seconds

The specific aerosols used in these studies were prepared as follows

1 Plutonium nitrate as $\text{PuO}_2(\text{NO}_3)_x$ is soluble in ethyl ether Advantage was taken of this property by introducing the plutonium nitrate into the exposure chamber as a spray Very small particles free from ether were produced by forcing the ether-plutonium solution through a gold jet after it had been allowed to come into equilibrium with carbon dioxide at a pressure of 760 pounds per square inch

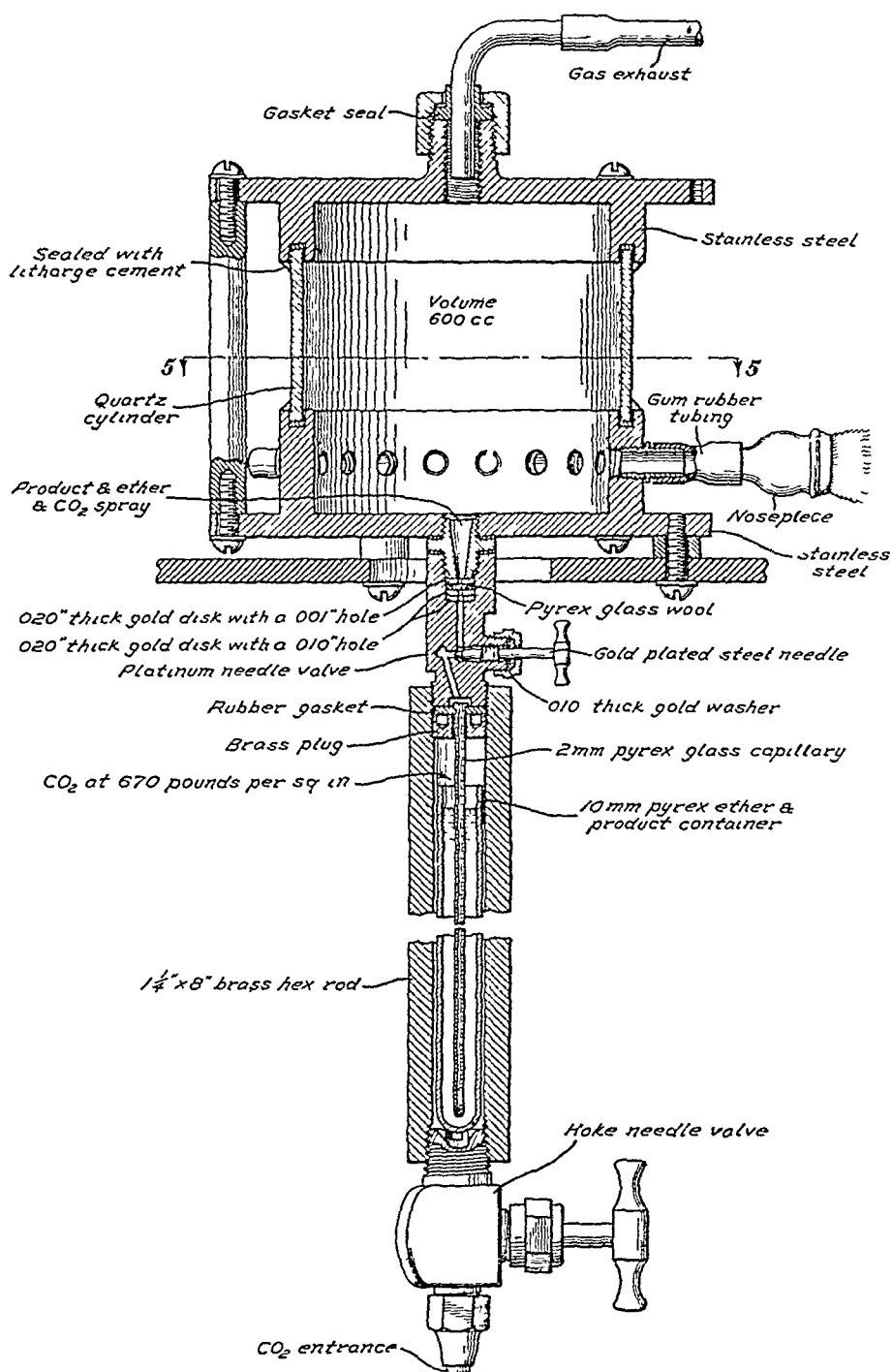


Fig 3—Diagrammatic sketch of the equipment used to produce aerosols of plutonium nitrate ($\text{PuO}_2[\text{NO}_3]_2$). The plutonium nitrate dissolved in ether, with carbon dioxide in the lower chamber, is forced through the gold jet at 760 pounds of pressure into the upper exposure chamber. As the ether evaporates in the warm chamber, the plutonium nitrate aerosol is suspended. Its droplet size is further reduced by the extreme effervescence of the carbon dioxide in the ether at atmospheric pressure.

The drawings in figures 2 and 3 were made by Mr. Gaines Heim.

(53 Kg per square centimeter) An effervescent ether-plutonium solution was produced when the jet was directed into the warm exposure chamber, the temperature causing the ether to evaporate. The plutonium yielded to the rat was about 10 per cent of the total plutonium used in the spray. The latter amounted to about 300 micrograms of plutonium per exposure. The exposure time was 3 to 4 minutes. The equipment devised to produce the plutonium nitrate aerosols is shown in figure 3.

2 Aerosols of plutonium in the plus four state as plutonium oxide (PuO_2) were produced by burning the plutonium salts and the plutonium metal itself. In the former instance, the solution was evaporated on graphite electrodes, and in the latter the metal was embedded in holes drilled in the centers of the 1/16 inch graphite rods. In both cases the electrodes plus the plutonium were burned in an atmosphere of oxygen in an electric arc. Two hundred micrograms of plutonium were burned for each exposure. The amounts yielded to the rats varied from 4 to 7 per cent of the material burned. The exposure consumed 30 to 60 seconds. One type of equipment used is shown in figure 4.

3 Aerosols of fission products occluded in uranium as uranium oxide (U_3O_8) and plutonium as plutonium oxide (PuO_2) were produced in a similar manner. At the time of exposure the radiations from the fission products predominated. The ratio of fission product disintegrations to the alpha particles arising from the uranium was about 20,000 to 1. The age of the fission products was 2 to 3 months. About 80 mg of uranium metal was burned at each exposure of 30 seconds' duration. The yield to the rats was about 1 per cent.

4 Carrier-free fission products were also prepared as an aerosol by evaporating the uranium-free solution on graphite rods and burning these. The solution of fission products used was 6 months to 1 year old. Owing to this fact, the fission products with short half-lives had decayed away. The fission elements remaining then were composed primarily of Sr^{89} and Sr^{90} , Y^{91} , Zr^{95} , Cb^{95} , Ru^{106} and Ru^{108} , Cs^{135} , Ce^{144} , and element 61¹⁴⁷.

5 Zr^{89} was used in a study of a human subject (one of us). The short half-life of this material, as well as the absence of long-lived radioisotopes, precluded any danger of excessive amounts of radiation to the subject. The active material was suspended as an aerosol in argon as follows. The Zr^{89} solution was evaporated on two concave gold buttons 1 cm in diameter. It was then dispersed in argon by passing 15,000 volts between two electrodes which were placed 5 mm apart. A 0.01 microfarad condenser was connected across the high voltage line. Oxygen was avoided in the chamber because of the production of undesirable amounts of ozone. Approximately 1 microcurie of Zr^{89} was inhaled by placing in the left nostril a short rubber tube which was also connected to the generator. The inhaled Zr^{89} was exhaled through the mouth into a glass wool filter, followed by several breaths of inactive air, so that the percentage of material exhaled could be calculated.

6 Carrier-free protoactinium, which has a 30 day half-life, was prepared as an aerosol by sparking evaporated Pa^{233} solution on a gold electrode in a high voltage electric discharge. This was administered to rats in the same manner as Zr^{89} .

Except in the Zr^{89} and protoactinium studies, the size of the particles produced by the aerosol generators was determined by collecting representative samples on amyl acetate films. The particle size was estimated with the aid of an electron microscope.

The lungs, kidneys, liver, spleen, skeleton, gastrointestinal tract, head, skin and the balance of the rat, as well as the separated excreta, were assayed for the radioactive material which was administered either as an aerosol or included

with the aerosol. These assays were performed on groups of 3 to 7 rats at periods as long as 256 days after exposure. A detailed report of the amounts of the active material deposited in various tissues of the rat as well as the metabolism of these elements is presented elsewhere⁵. Only values obtained for the head, lung and skeletal deposition, the daily rate of excretion, the particle size and the radioautographic studies are included in this report.

RADIOAUTOGRAPHIC TECHNIC

At intervals, as stated in the text, following the inhalation of the aerosol being studied, the rats were killed with chloroform. The lungs were fixed while intact

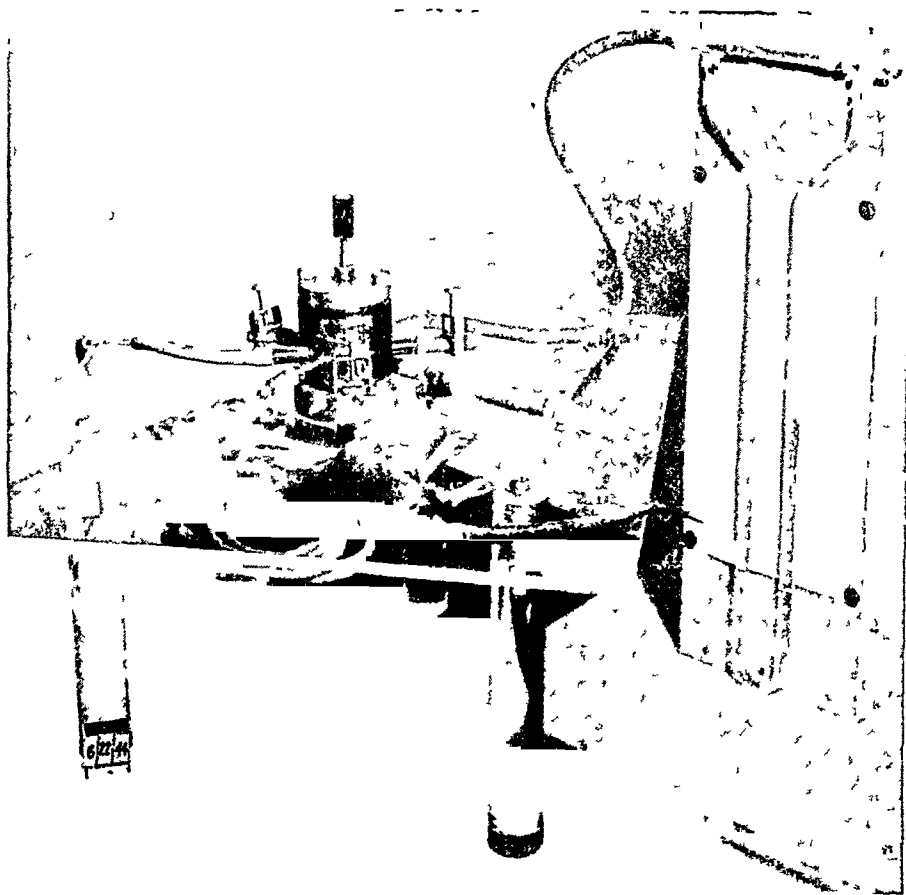


Fig 4—Photograph showing the complete aerosol exposure system. The anesthetized rat is breathing oxygen, at a rate illustrated by the flowmeter, along with the active aerosol produced in the electric arc.

in the animals by injecting 80 per cent alcohol (p_H approximately 8) into them through the trachea. Zenker-formaldehyde solution could not be used as a fixative, because it was found to cause fogging of the x-ray film. Formaldehyde solution U S P could not be used, because it leached plutonium from the tissues. After 24 hours' fixation, the lungs were dehydrated in dioxane and embedded in paraffin. Sections 10 microns in thickness were cut and mounted on microscopic slides. The paraffin was removed with xylene. A thin protective covering of celloidin was used on the section. Each slide was placed in contact with a

piece of "no screen" x-ray film and wrapped in black paper to exclude light. Lead weights were used to press the film and the tissue together. The details of the procedure used have been reviewed elsewhere by Axelrod and Hamilton⁶. After the films were exposed and developed, the lung sections were stained with hematoxylin and eosin. Photomicrographs of representative slides and their radioautographs are presented in this report.

RESULTS

The aerosols studied here were, in general, deposited in the same respiratory areas of the rats despite their rather different origin and physical and chemical properties. Immediately after exposure the major proportion of them were found to be distributed from the nasopharyngeal region down through the bronchial tree and into the alveoli of the lungs. The rate at which this general region functioned in the removal of the aerosols was composed of two very different time intervals. The presence of ciliated epithelial cells allowed almost complete removal of the active particles in a matter of hours. This relatively rapid removal of material deposited on the ciliated epithelium agrees with the ciliary removal rates reported by Proetz⁷. In the regions of the lungs, such as the ductus alveolaris and the alveoli, the removal of any large percentage of an aerosol consumed a range of time of the order of many months. The route of removal was primarily via the bronchial tree. Since plutonium and the majority of the fission products given are not absorbed in the gastrointestinal tract to any extent, the material excreted via the bronchial tree could be detected in the fecal fraction of the excreta^{5a, c}. However, the products of fission which are comparatively recent contain appreciable amounts of Ba¹⁴⁰, which has a half-life of 12.5 days. In addition, all the solutions contained Sr⁸⁹ and Sr⁹⁰, which have 55 day and 25 year half-lives, respectively. As shown by Hamilton,^{5c} these two elements would have been absorbed to some extent through the lungs had they been in solution, since they are absorbed from the intestinal tract when administered orally. The absorbed portion amounted to from 5 to 60 per cent of the total amount given. Of the absorbed portion, 65 per cent was found deposited in bone. It follows, then, that a low bone deposition of these fission products suggests that they were locked in insoluble particles when administered as aerosols. In these studies the insoluble particles could have been carbon, plutonium or uranium in combination with their oxides.

Plutonium Nitrate ($\text{PuO}_2[\text{NO}_3]_2$) Sprays—Table 1 shows the fate of plutonium nitrate when administered to rats as a spray, the plutonium being in the plus six state. At 10 minutes after exposure the lungs and the head, the latter including the region above the trachea, contained approximately the same amount of aerosol. It can be seen that the plutonium was removed from the head quickly and was found in the fecal fraction. After one day the lungs continued to lose plutonium, but at a rate much more slowly than the head. The plutonium which was transported across the capillary barrier of the alveoli by whatever means eventually found its way to bone. This transport was not a great factor in the elimination of the pulmonary plutonium, since the highest value obtained was 12 per cent of the total 64 days after administration. The total fraction of plutonium absorbed from the lungs was probably of the order of 20 per cent of the amount retained in the respiratory tract, since 60 to 70 per cent of plutonium absorbed after intramuscular injection is deposited in bone.

⁶ Axelrod, D. J., and Hamilton, J. G. The Radioautographic Technique, U. S. Nav. M. Bull., (Supplement) March-April 1948, p. 122.

⁷ Proetz, A. W. J. Laryng. & Otol. 49: 557, 1934.

In addition, the absorption of plutonium occurred promptly, since there was little additional plutonium deposited in bone after the fourth day. The aerosols were removed from the head and the lungs by being passed up the bronchial tree, since they were detected primarily in the feces. This includes the fraction that was initially deposited on the ciliated epithelium of the upper respiratory tract and was rapidly swept out, as well as that retained and slowly eliminated from the alveoli.

Radioautographic studies were made at 10 minutes and 16 days after exposure. At the 10 minute interval the ciliated passages of the lungs, as well as the alveoli, contain deposits of plutonium (fig 5A). At 16 days, however, deposition appears to be primarily in areas included in the ductus alveolaris and its primary lobules (fig 5B). The data shown in table 1 demonstrate a reduction of plutonium for all areas of the lung.

Plutonium Oxide Aerosols Prepared from Plutonium Metal and Salts of Plutonium—Plutonium oxide particles were produced by burning plutonium with graphite in an electric arc in an atmosphere of oxygen. In this case plutonium was presented to the rats in the plus 4 state and probably as some plutonium metal. Its chemistry in the plus 4 state is somewhat similar to the lanthanide series of rare earths. This is in contrast to the plus 6 state of plutonium resulting when the plutonium nitrate sprays were used. The latter compound, $\text{PuO}_2(\text{NO}_3)_2$, is more

TABLE 1—*Fate of Plutonium Inhaled as Plutonium Nitrate Spray*

	Percentage of Total per Organ at Given Time After Exposure of Rats				
	10 Min	1 Day	4 Days	16 Days	64 Days
Lungs	48	39	28	21	9
Head	37	4	12	08	01
Bone	5	8	11	79	12
Daily rate of elimination		23	3	09	03

similar in chemical characteristics to uranyl salts of uranium. The particle sizes produced by the electric arc varied from 0.06 to 38 microns. Their average size was 0.49 micron (fig 6).

Tables 2, 3 and 4 summarize the results obtained with plutonium oxide aerosols. It can be seen that the results are similar to those obtained with the plutonium nitrate sprays with the exception that relatively little of the aerosol was absorbed into the blood stream, as evidenced by minimal deposits in the bones. Consequently, less was found in the organ of primary deposition, bone. Plutonium oxide aerosols are gradually removed from the nonciliated areas via the bronchial tree. This removal takes a much longer time than that of the aerosols deposited in the portions of the respiratory tract above the trachea, which are included in the head fraction, as well as those deposited on the ciliated epithelium of the bronchial tree. The plutonium oxide aerosols are eliminated in the same manner and at comparable rates whether they are produced by the burning of plutonium salts or by the burning of the metal.

The actual amount of plutonium administered to the rats used in these studies varied from 1 to 3 micrograms per animal. This amount would have given the lungs of the rats about 2 roentgens (r) per day. In this case the electron microscope pictures suggest that some of the aerosol existed as the metal. Furthermore, it is worthy of attention that the aerosol particles appear to follow the same path whether they are detected by following the alpha activity of the plutonium or the beta and gamma radiations of the fission products incorporated in the plutonium.



Fig 5A—Radioautograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium chloride and plutonium nitrate. The rat was killed ten minutes after exposure. Plutonium is heavily deposited on ciliated bronchial surfaces and in alveoli ($\times 6$)

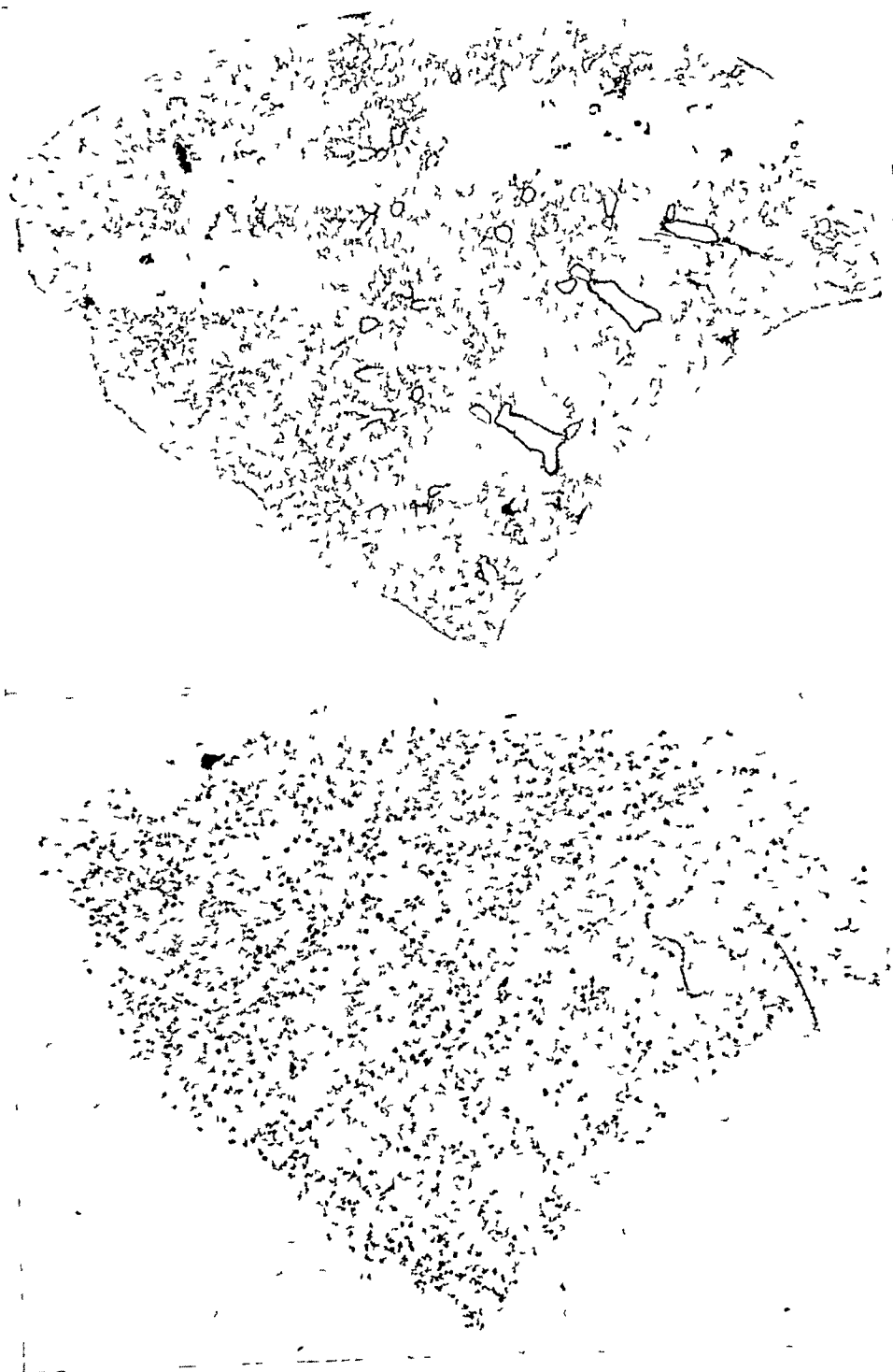


Fig 5 B—Radiograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium chloride and plutonium nitrate. The rat was killed sixteen days after exposure. Plutonium has been almost completely cleared off of the ciliated surfaces and is located in alveolar structures ($\times 10$)

particles. These results are in agreement with our view that the particles themselves were being followed rather than any one element or group of elements, since the handling of fission products of this age would have been different from that of plutonium (also the carrier-free fission products mentioned on an earlier page⁵). Less than 2 per cent of the total material administered as plutonium was absorbed through the lungs to enter the blood stream and be deposited in the skeleton. With respect to the fission products, the amount thus absorbed was about 7 per cent.

Radioautographic studies suggest that the alveoli were the major area of retention of the inhaled plutonium fission product aerosols after the material laid down on the ciliated passages of the lungs had been expelled (figs 7 A, B, C and D). At the time of exposure 10 fission product disintegrations were occurring for each alpha particle emitted from the plutonium. The plutonium was prepared by placing plutonium metal in graphite electrodes. These were placed in the deuterium oxide pile at the Argonne National Laboratories. At the time of the production of the aerosols the fission products had an average age of 2 months. We estimate

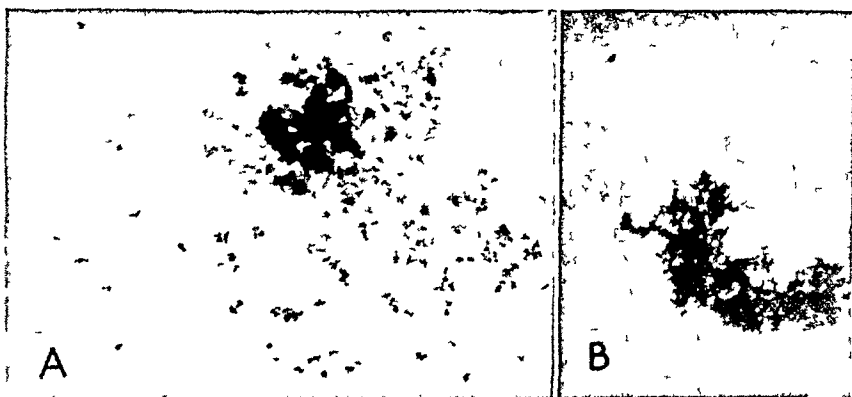


Fig 6—Electron micrographs of plutonium oxide aerosols produced by burning plutonium metal on graphite electrodes in an electric arc ($\times 8,000$)

that 25 per cent of the film darkening was due to alpha particles and 75 per cent to fission product particles.

Aerosols Composed of Uranium Oxide Containing Fission Products—Aerosols similar to those composed of the plutonium oxide fission product particles just discussed were produced by burning uranium wire in an electric arc. Smoke particles produced in this manner would be quite comparable to those which might arise from accidental destruction of a uranium pile. The uranium wire had been previously placed in the Clinton pile at Oak Ridge, Tenn, and the fission products were produced by prolonged neutron irradiation. About 60 days elapsed between the time of their removal from the pile and the production of aerosols for inhalation. Although the particle sizes produced on burning ranged from 0.06 to 9.4 microns, the average particle was larger than those produced from plutonium by about a factor of 2, being 0.97 micron in size (fig 8). This increase in particle size is apparently reflected in the relatively greater entrapment in the respiratory structures of the head. This greater deposition observed in the head as compared with the lungs agrees with the observations of Barclay and co-workers,⁸

⁸ Barclay, A. E., Franklin, K., and MacBeth, R. G. *Am J Roentgenol* 39: 673, 1938.

who showed that bismuth carbonate dust particles 2 to 12 microns in size were not deposited past the respiratory bronchioles and with those of Policard,⁹ who showed that silica and coal particles larger than 10 to 12 microns were not found in the alveoli of miners. Table 5 summarizes the results obtained by exposing

TABLE 2—*Fate of Plutonium Inhaled as Plutonium Oxide Aerosols Made by Burning Plutonium Metal Embedded in Graphite*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats				
	10 Min	1 Day	4 Days	16 Days	64 Days
Lungs	37		42	20	11
Head	58.2		3	0.3	0.1
Bone *	1		0.5	0.4	0.4
Daily rate of elimination		23.7	0.1	0.4	<0.001

* The relatively higher values obtained for bone 10 minutes after exposure are not considered to be significant, since there is a possibility that the animals were contaminated with the aerosol deposited on the nose and face.

TABLE 3—*Fate of Plutonium Inhaled as a Plutonium Oxide Aerosol Prepared by Burning Plutonium Metal Embedded in Graphite*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats					
	10 Min	1 Day	4 Days	16 Days	64 Days	256 Days
Lungs	34	35	27	29	12	4.8
Head	59	13	0.2	0.2	0.5	0.01
Bone *	1	3	1	0.8	1.5	0.2
Daily rate of excretion		28.5	6.4	1	0.2	0.01

* The values obtained for the skeleton could have been high because of outside contamination of the skeleton occurring when the rats were dissected at 10 minutes, 1 day and 4 days after exposure.

TABLE 4—*Comparative Fate of Plutonium Oxide Aerosols Containing Products Created by Fission of Plutonium Metal in a Deuterium Oxide Pile*

	Percentage of Total Administered per Organ at Given Time After Exposure of Rats							
	10 Min		1 Day		4 Days			
	As	Pu	As	F P	As	Pu	As	F P
Lungs	41		43			61		56
Head	36		40			2.4		2.4
Bone *	2		3			2		7
Daily rate of excretion				19	23	0.5		4
	16 Days		64 Days		128 Days			
	As	Pu	As	F P	As	Pu	As	F P
	As	Pu	As	F P	As	Pu	As	F P
Lungs	16		17		8	9	15	9
Head	0.3		0.3		0.2	0.1	0.1	0.3
Bone *	2		2		0.01	0.3	0.01	0.4
Daily rate of excretion	0.3		0.9		0.2	0.2	0.5	0.1

* The values obtained for the skeleton could have been high because of outside contamination of the skeleton occurring during dissection of the rats at 10 minutes, 1 day and 4 days after exposure.

rats to uranium aerosols. It can be seen that the elimination of the aerosol is via the bronchial tree and that the rate of elimination is comparable to that of plutonium aerosols. Relatively small amounts of fission products were absorbed and deposited

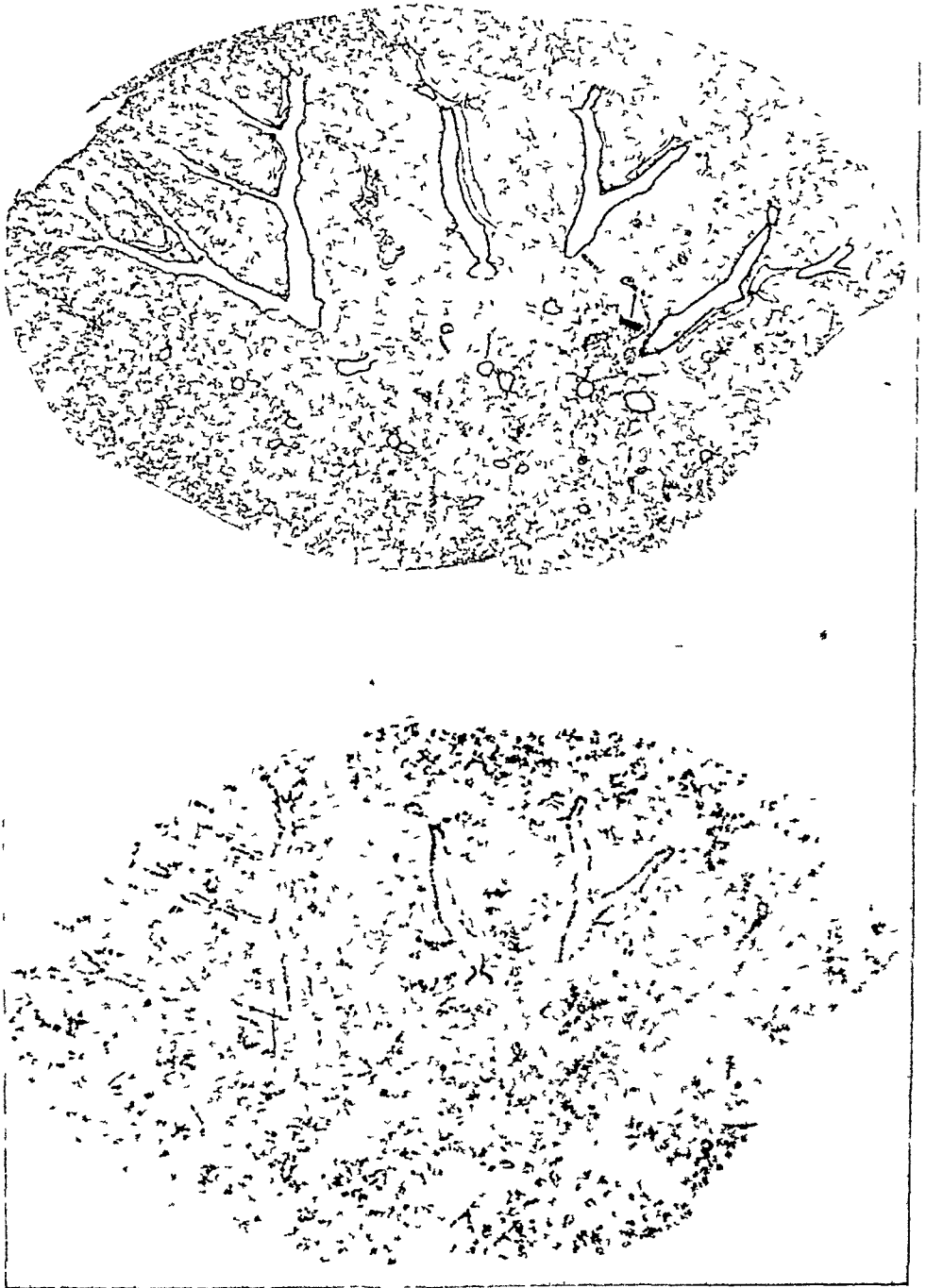


Fig 7 A—Radioautograph (lower) and lung section (upper) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium metal. The rat was killed ten minutes after exposure. The material is deposited on ciliated surfaces and alveoli ($\times 68$)



Fig 7 B—Radioautograph and lung section (superimposed) showing pulmonary deposition of plutonium oxide aerosol obtained by burning plutonium metal. The rat was killed ten minutes after exposure. The tissue section was mounted directly on alpha particle plates. The photomicrograph shows the alpha tracks from plutonium deposited on the walls of two bronchi and on the alveolar epithelium ($\times 290$).

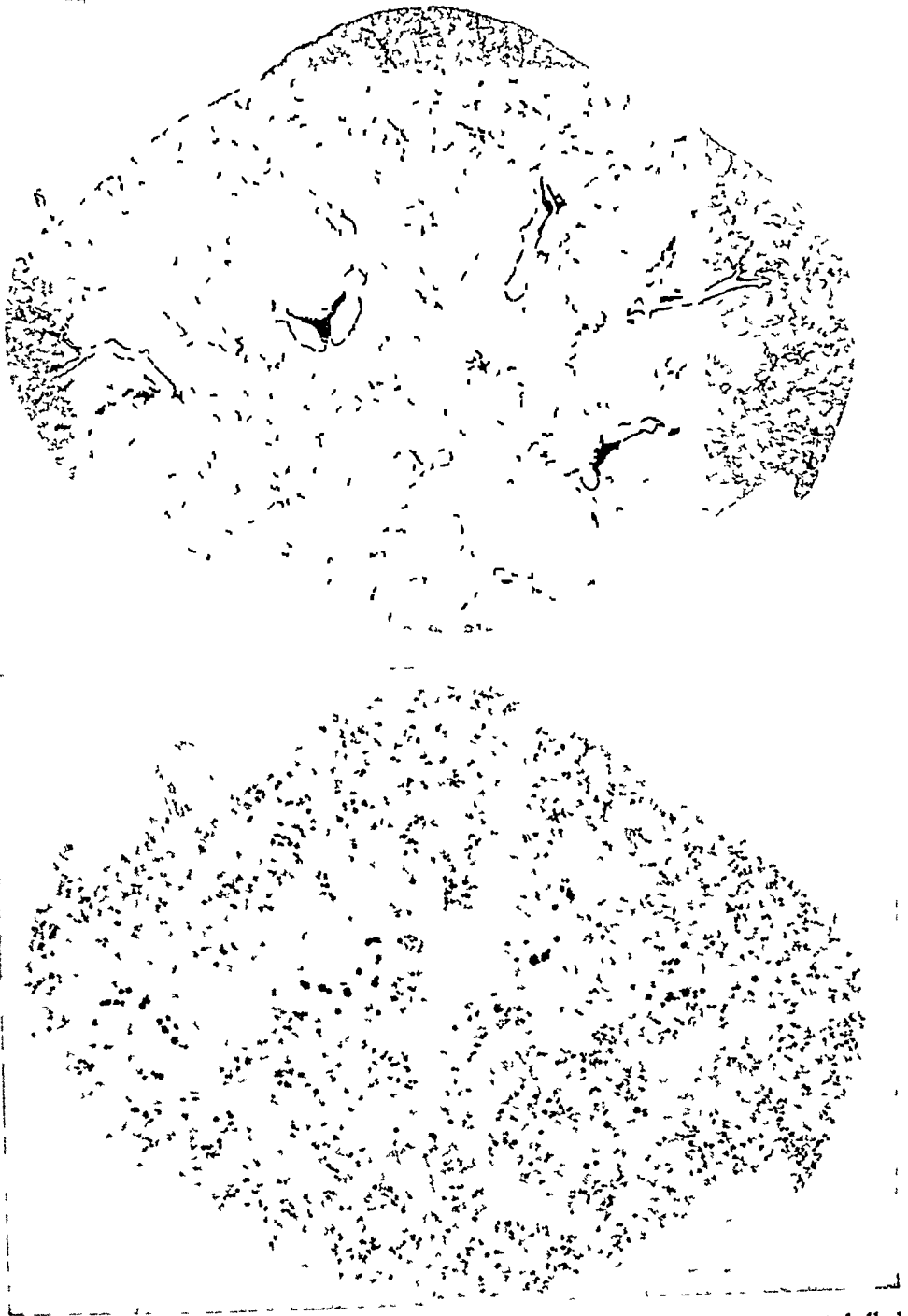


Fig 7 C—Radioautograph (lower) and lung section (upper) from rat killed one day after inhalation of plutonium oxide aerosol obtained by burning plutonium metal. No plutonium remains on the surfaces of the bronchial tree, but it is arranged in spots throughout all of the alveolar structure ($\times 75$)

in the skeleton. These results are in contrast with those obtained when solutions of fission products are admitted to the lungs by intubation. About 0.1 mg of uranium was deposited in the rats along with the fission products inhaled as an aerosol.



Fig 7 D—Radioautograph (lower) and lung section (upper) from rat killed sixty-four days after inhalation of plutonium aerosol obtained by burning plutonium metal. A considerable amount of plutonium remains in the lung scattered throughout the alveolar structure. ($\times 8$)

This amount of uranium produced no apparent toxic effect, the amount of radiation arising from the fission products was below the accepted tolerance level of 0.1 r per

day In this case the percentage of fission products deposited in bone was much greater 4, 16 and 64 days after administration, as can be seen from an examination of table 5

This suggests that the uranium oxide particles occluded the fission products and that detection of the fission products was in reality detection of uranium oxide particles

The radioautographic studies shown in figures 9A and 9B demonstrate the areas of pulmonary deposition at 10 minutes and at 1 day after exposure to aerosol of uranium oxide containing fission products The areas of deposition are the same as those previously noted, primarily the alveoli after removal of material deposited on ciliary epithelium

The aerosols mentioned previously have been composed primarily of the oxides of plutonium, the oxides of uranium, fission products and plutonium nitrate. Additional studies were performed in which the active particles were fission products in the carrier-free state, thus they contained relatively little mass and no plutonium or uranium.¹⁰ Since they were burned with graphite electrodes, the only particulate matter incorporated with the fission products was carbon The actual number of radioactive atoms which were traced was relatively small, less

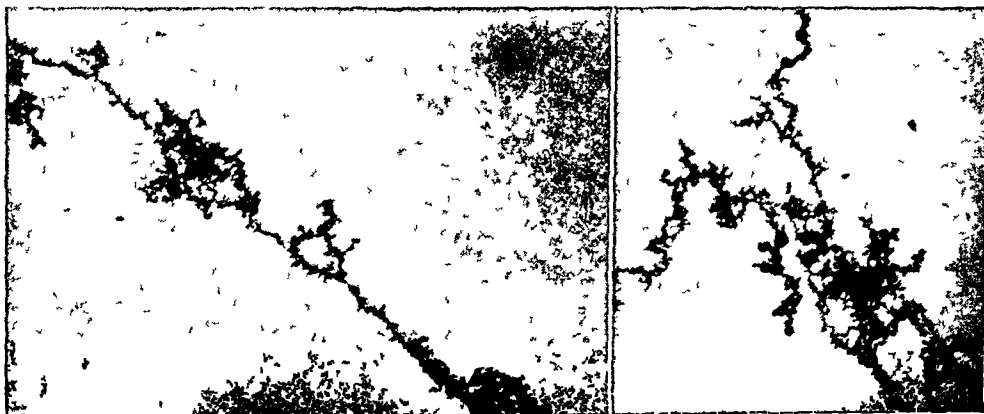


Fig 8—Electron micrographs of fission product aerosols prepared by burning uranium metal plus fission products in graphite electrodes in an atmosphere of oxygen ($\times 8,000$)

than 10^{18} atoms to each rat With respect to plutonium, 1 to 3 micrograms was deposited in the lungs of rats during the exposure About 0.1 mg of uranium was administered with the fission product uranium oxide aerosols studied Since the major part of the fission products produce insoluble oxides on burning, they probably remained incorporated with the carbon particles themselves, although this is not certain, they were not disposed of by the lungs in the same manner as fission products admitted to the lungs by the tracheal intubation previously cited The data obtained on the deposition and the fate of aerosols of this nature are summarized in table 7

Relatively small amounts of fission products were absorbed into the body and deposited in bone This statement of course must be qualified with respect to the age of the fission products used In this case the fission products were 2 to 3 months old when incorporated with plutonium and uranium aerosols The fission products used in the carrier-free state were from 6 months to 1 year old For

¹⁰ The carrier-free fission products were prepared at the Clinton Engineering Works, Oak Ridge, Tenn

this reason of age, the most abundant fission products which were present when the radioactive assays were made included strontium, yttrium, zirconium, columbium, ruthenium, cesium, cerium and element 61. Ruthenium and cesium were found to be rapidly eliminated and not retained by the skeleton. The major portion of the material was eliminated via the bronchial tree. The average size of the particles was found to be 0.63 micron and representative pictures are presented in figure 10.

TABLE 5—*Distribution of Carrier-Free Fission Products Administered as a Chloride Solution by Pulmonary Intubation*

	Percentage Remaining in Whole Organ at Given Number of Days After Administration		
	4 Days	16 Days	64 Days
Lungs	40	21	7
Trachea	3	0.1	0.2
Bone	35	61	74

TABLE 6—*Fate of Uranium Oxide Aerosol in Rats When the Uranium Oxide Particles Are Followed by the Determination of Radioactivity of the Included Fission Products*

	Percentage of Total Administered per Organ at Given Time After Exposure				
	10 Min	1 Day	4 Days	16 Days	64 Days
Lungs	12	10	5	7	4
Head	82	7	<0.01	<0.01	0.08
Bone	0.5	1.4	1.9	1.3	1.0
Daily rate of excretion		42	3	5	0.1

TABLE 7—*Fate of Aerosols Composed of Graphite and Carrier-Free Fission Products When Burned in an Electric Arc in an Atmosphere of Oxygen and Inhaled by Rats*

	Percentage of Total Aerosol Administered per Organ at Given Time After Exposure				
	10 Min	1 Day	4 Days	16 Days	64 Days
Lungs	65			25	6
Head	15.0			<0.01	<0.01
Bone	1.5			7.0	7
Daily rate of excretion		30	4	0.8	0.2

In addition to the studies described to this point, a series of rats was exposed to protoactinium. This radioelement, Pa^{233} , in the carrier-free state, was administered to rats as an aerosol by driving it off of gold electrodes in an electric arc in an atmosphere of argon and, of course, eliminating the carbon in the aerosol. Less than 0.001 microgram of protoactinium was administered to these rats, and it was presumably in the form of protoactinium oxide (Pa_2O_5). However, this material could have combined with particulate material and/or bacteria, the presence of which has been demonstrated in the lung by Jones.¹¹ At any rate, protoactinium, even though in small amount, is not absorbed into the blood stream from the lung.

¹¹ Jones, F. S. J. Exper. Med. 36:317, 1922.

to any great extent. The results obtained on protoactinium are summarized in table 8.

As with the aerosols discussed earlier, protoactinium aerosols produced in the manner described in the foregoing paragraph are not absorbed into the body from



Fig 9 A—Radioautograph (lower) and tissue section (upper) showing the pulmonary deposition of fission product aerosol obtained by burning uranium metal plus fission products. The rat was killed ten minutes after exposure. The fission product mixture was deposited on ciliated surfaces and throughout the alveolar structure ($\times 68$).

the lungs but are eliminated rapidly from the head and more slowly from the lungs via the bronchial tree.



Fig 9 B—Radioautograph (lower) and tissue section (upper) showing pulmonary deposition one day after exposure to fission products from burning uranium metal plus fission products. The material is almost completely cleared from ciliated surfaces and is scattered throughout the alveolar structure ($\times 8$)

It is of interest to know how much of an aerosol is retained during one inspiration and how much is eliminated in the following expiration. It is realized, of course, that the size of particles, the experimental subject used and the velocity of the pulmonary air currents will also modify the results. Some information was obtained from rats exposed under the experimental conditions noted in the section

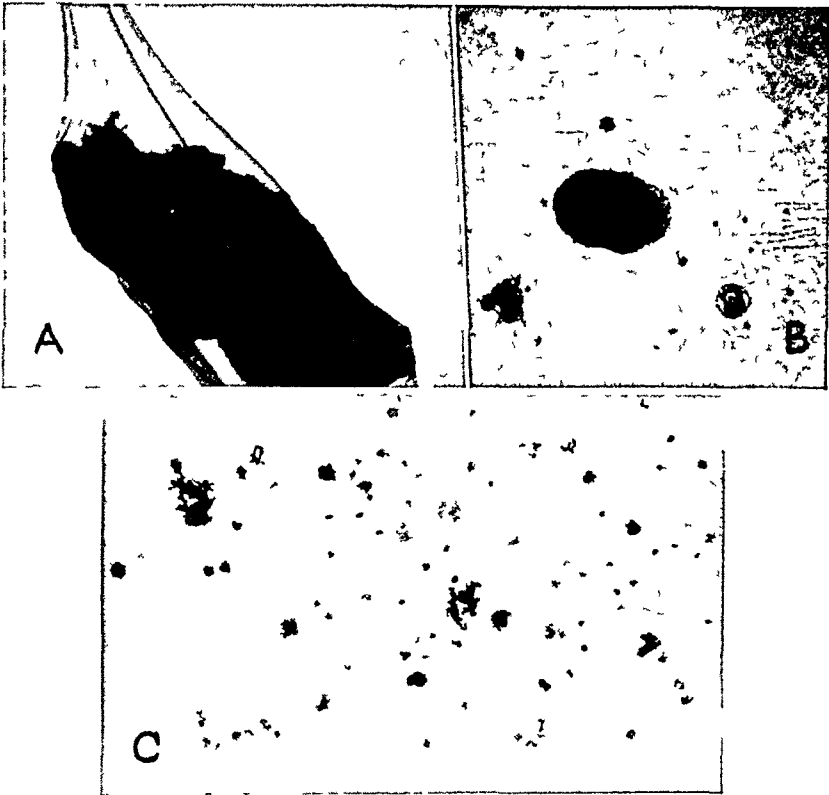


Fig 10—Electron micrographs of aerosols of carrier-free fission products evaporated on graphite rods and burned in an electric arc (× 8,000)

TABLE 8—*Fate of Protoactinium Aerosols When Produced by High Voltage Electric Discharge Between Gold Electrodes in an Atmosphere of Argon and Inhaled by Rats*

	Percentage of Total Aerosol Administered per Organ at Given Time After Exposure				
	10 Min	1 Day	4 Days	16 Days	64 Days
Lungs	42		46	39	7
Head	39		08	04	04
Bone	03		31	09	11
Daily rate of excretion		25	2	05	05

under "Methods of Study" by constructing a valve system which allowed the material exhaled by the rat to be collected separately from that inhaled. When plutonium oxide particles were used, 63.4, 87.6 and 66.5 per cent of the total aerosol inhaled was initially retained in the animal. The particles exhaled were 0.5 to 1.0 micron in diameter, and representative electron micrographs are presented in figure 10.

Similar studies were done on rats and on one of us, carrier-free Zr^{89} with a half-life of 78 hours being used. The Zr^{89} was dispersed as an aerosol, probably as ZrO_2 , by the use of the electric discharge in argon described earlier in the protoactinium experiments. Although the material was carrier free, it was not entirely free of foreign particles. These traces of foreign material were introduced while Zr^{89} was being chemically separated from the target material used, which was deuterium-bombarded yttrium oxide. In the single human study done, all but 2.2 per cent of the activity inhaled was retained. Thirty-two per cent was removed via the bronchial tree in 71 hours. This left 66.7 per cent of the activity estimated to have been given remaining in the lungs, presumably in the alveoli.

The parallel experiments in rats showed that 24 per cent of the zirconium aerosol was deposited in the lungs and 40.9 per cent in the head, 28.8 per cent was exhaled and not retained. After 3 days, the lungs contained 18.9 per cent and the head 2.5 per cent, and 60.6 per cent had been eliminated via the bronchial tree, while 3.3 per cent of the Zr^{89} apparently had been absorbed and deposited in the skeleton.

COMMENT

The preceding experiments demonstrate that particulate material 1 micron in size and smaller, composed of plutonium or uranium and/or their fission products, which include a large series of elements in the central region of the periodic table, as well as protoactinium, are deposited in the alveolar structures of the lung, as well as in the air-conditioning passages of the lungs containing ciliated epithelium. This fact is borne out by the radioautographic evidence presented, as well as by the different elimination rates of ciliated epithelium as compared with other structures of the lung, including the ductus alveolaris and beyond. Despite their different chemical and physical natures, the aerosols used in these studies were eliminated from the lung primarily via the bronchial tree at comparable rates. Although the mechanism by which these aerosols were eliminated from the lung was not the problem at hand in these studies, quantitative data were obtained demonstrating their removal and qualitative data indicating their areas of deposition. It would appear to us that the aerosols used, under the experimental conditions of these studies, are removed via the bronchial tree, which is one of the routes suggested by Carleton¹² and others¹³.

No evidence was obtained that any large percentage of the material used in these studies found its way to the lymphatic system of the lungs. In fact, this appears unlikely, since the particles inhaled were continuously removed via the bronchial tree as long as 8 months after exposure. This suggests that the particles remained on the air side of the alveolar structures. The mechanism of their removal appears to be

12 Carleton, M. H. *J Hyg* **22** 438, 1923-1924.

13 Gardner, L. U. *Am Rev Tuberc* **4** 734, 1920-1921. Lemon, S. W., and Higgins, G. M. *ibid* **28** 470, 1933. Drinker, C. K. *J Indust Hyg* **3** 295, 1922. Pennar, H. H. *J M Research* **42** 9, 1920.

the same with respect to the aerosols studied here. This can be demonstrated by plotting all of the pulmonary elimination data on one curve. This is presented in figure 11. Any of the data presented in this report would fall on this curve despite the difference of the materials used in the production of the aerosols.

SUMMARY

The purpose of these experiments was to ascertain the possible hazard resulting from inhalation of fissionable materials and fission products. Aerosols of plutonium, uranium plus fission products, and protoactinium were administered to rats. A Zr^{89} aerosol was administered to a human subject and to rats. Aerosols of the aforementioned elements were almost totally retained by the head and the lungs immedi-

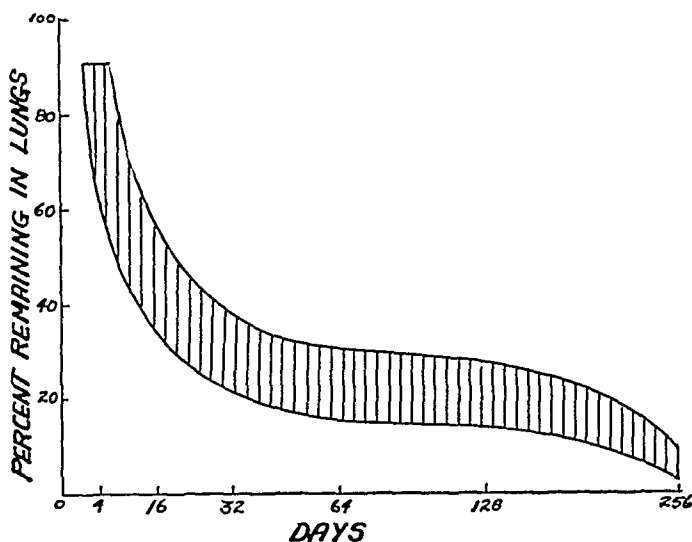


Fig 11—Elimination of inhaled radioactive aerosols. The graph represents the data obtained from rats which had inhaled aerosols containing plutonium (in the plus 4 and plus 6 state), uranium, plutonium fission products, uranium fission products and carrier-free fission products.

ately after exposure. After four days the lungs contained the largest percentage of these elements. The elements deposited in the head and bronchial tree were quickly eliminated via the gastrointestinal tract. The same avenue of elimination was presumably used by the nonciliated portion of the lungs, but at a slower rate. The small percentage absorbed into the body was primarily deposited in the skeleton after conditions of equilibrium had been established. Radioautographic studies indicate that the pulmonary site of deposition of these materials is in the bronchial passages and the alveolar structures. The materials are rapidly removed from the bronchial tree, presumably by ciliary action, and are slowly released from the alveoli. No accumulation of any of the radioelements was observed in either blood vessels or lymph nodes.

NEW ELASTIC FIBERS FORMED IN THE WALL OF THE GALLBLADDER IN DISEASE OF THE GALLBLADDER

Pathologic, Clinical and Roentgenologic Data Concerning
a Hitherto Neglected Lesion

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IT IS A well known histologic fact that, compared with the respiratory and the anterior digestive tract, the posterior alimentary tract is poor in elastic tissue. The gallbladder, a derivative of the intestine, does not stand as an exception. According to Maximow and Bloom¹ and Hass,² there is normally a network of elastic fibers which accompanies the smooth muscle bundles and, also, there are a few elastic fibrils elsewhere, particularly in the perimuscular connective tissue layer. However, in my experience the said network and fibrils are to be seen only under high magnification, at least in the human species. At lower magnification, namely, less than $\times 60$, only the elastica associated with the vessels and the peritoneal lining can be perceived.

The scarcity of elastic fibers may explain why pathologists have apparently failed to investigate its morbid variations. To my knowledge, new formation of elastica in the wall of the gallbladder has been mentioned for the first time only recently, namely, in the second edition of the Chiray, Pavel and Lomon³ treatise "La vésicule biliaire et ses voies d'excrétion," issued in 1936. Lately I⁴ have published more detailed studies on the same subject. The newer textbooks are entirely silent on the matter.

However, disease of the gallbladder is often attended with definite changes in the amount of elastin. In old hydrocholecystis fibrous degeneration leads finally to diminution and disappearance of elastin.

From the Institute of Pathological Anatomy, University of Montreal

1 Maximow, A. A., and Bloom, W. A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1939

2 Hass, G. M., Arch., Path. **27** 334, 1939

3 Chiray, M., Pavel, L., and Lomon, A., in Chiray, M., and Pavel, I. La vésicule biliaire et ses voies d'excrétion, ed 2, Paris, Masson & Cie, 1936, pp 335-336

4 Riopelle, J. L. J. de l'Hôtel-Dieu de Montreal **12** 1, 1942, Union med du Canada **75** 1486, 1946

In other conditions, on the contrary, there is a definite increase in elastica, so that suitable staining methods show deposits of that substance even at low magnification

It is the purpose of the present paper to bring forward new data on the increase of elastic tissue in the wall of the gallbladder and to discuss anew its genesis and evolution

MATERIAL AND METHODS

The present paper is based on a study of 1,017 cases of disease of the gallbladder, a number which represents nearly all the cases in which cholecystectomy was done in the Hôtel-Dieu de Montreal, in the years 1944, 1945, 1946 and 1947. The specimens were generally emptied of their contents immediately after their removal and allowed to retract freely. They were afterward incised lengthwise and fixed in Bouin's fluid except in rare instances in which formaldehyde solution or formaldehyde-Muller fluid was used. Stretching or kinking of the viscus was avoided. Length was measured after fixation, while thickness of the wall was computed on histologic sections. Three whole transverse sections were made, in each third of the gallbladder. Tissue blocks were embedded in paraffin. Sections were routinely stained for elastin by Weigert's resorcin-fuchsin technic and counterstained with hemalum, phloxine and saffron. Orcein and Verhoeff's ferric perchloride stain were occasionally used. Out of the general material, series of diverse lengths were taken, for statistical purposes. Histologic selection of cases was done without knowledge of age or sex of patients, or of any other data.

PATHOLOGIC ANATOMY OF NEW GROWTH OF ELASTIC TISSUE

In diseased gallbladders one often finds notable increases of elastica in various locations, namely, in the peritoneal layer of the viscus, in its vessels and, lastly, in the wall proper, outside the vessels.

Thickening of the elastic network of the peritoneum of the gallbladder is frequent in disease of the gallbladder, especially in chronic inflammation. However, this is not peculiar to the serosa of the gallbladder and does not seem to correlate with elastic fiber changes in other layers. For these reasons, I have here only mentioned its existence.

New elastic fibers may also be formed in walls of the blood vessels chiefly in the arteries. Their formation is in no way different from that usually seen in vascular disease, particularly in endarteritis obliterans. This lesion is often most conspicuous in old cholecystitis. There is not any close connection between it and extravascular increase of elastica, as the latter appears much earlier than the former.

Last in line but most important is interstitial production of elastica in the wall of the viscus. The cause, genesis and structure of this increase are peculiar to the gallbladder, so that they deserve special consideration.

There are many modalities of parietal increase of elastic tissue according to distribution and relationship to preexisting structures.

The first variety is seen in more or less localized destruction of the wall, with scar tissue replacement. This is usually the case when calculi are impacted at the neck of the gallbladder, where the mucosa is commonly ulcerated and the rest of the wall is totally or partially replaced by fibrous tissue. In this fibrous tissue one often sees amputation neuromas, hyperplastic endarteritis and considerable



Fig 1—*A*, half of a transverse section of the body of the gallbladder, Weigert's resorcin-fuchsin, hemalum, phloxine, saffron, $\times 25$. The mucosal folds are still preserved, although flattened. Numerous elastic deposits are visible in the muscle bundles of the media. A compact wavy band of elastica is seen in the perimuscular tissue layer. In the lower part of the figure the individual elastic fibrils are separated by hyalinized collagen.

B, progressive disappearance of mucosal folds. Moderate hypertrophy of the muscle coat with excess of elastica in a few muscle bundles. Weigert's resorcin-fuchsin, hemalum, $\times 45$. A thin, oblique musculoelastic fascicle joins the media with the perimuscular elastic layer. Aberrant fascicles may be incorporated later to the new-formed elastic layer after disappearance of the muscle fibers. Note the partial hyalinization of the elastic layer.

deposits of elastic substance In old cases the elastic fibrils curl up in dense, felt-like masses and lose part of their affinity for orcein The observations that these deposits occur in a destructive lesion, have no organoid structure and show progressive loss of specific tinctorial affinity for orcein point to the degenerative nature of the change, which is reminiscent of elastic degeneration of the skin

In most other instances the new elastica shows good affinity for its specific stains, as well as an organoid structure, so that it can hardly be considered as degenerative

New fibrils may appear more or less diffusely in the common connective tissue of all layers However, such increase of elastica is slight and can be seen only at high magnification Its existence or importance is hard to assess in individual cases, owing to the present ignorance of the normal variations according to age, sex and other factors, such as preexisting inflammation

More important deposits of elastica may be seen at low magnification in specialized connective tissue or in mesenchymal derivatives They are indeed observed either in the smooth muscle bundles of the tunica media or in the perimuscular connective tissue layer of the outer coat, a layer which is anatomically and functionally associated with the tunica muscularis Here the deposited elastica, be it diffuse or in multiple separate foci, is not only organoid but also systematized, that is, confined to definite places As it can easily be seen and graded at low magnification, it is possible to correlate it with various clinical or roentgenologic data

In the muscle coat the new deposits of elastica assume various forms The most frequent consists in a thickening and multiplication of the normally minute fibrils coursing along the surface of the smooth muscle cells Normal or thin muscle fibers persist in the densified elastic network Usually the change affects a whole muscle bundle along considerable lengths, so that it is seen with elective stains as a black-brown streak running in the middle layer This change may appear in isolated bundles, or it may affect the greater part of the muscle coat of the gallbladder (fig 1)

More rarely, the muscle bundles or the whole thickness of the tunica muscularis is locally interrupted through total disappearance of its fibers Then, its continuity is preserved only by one or many thin streaks of elastica, which is nothing more than the persisting and hypertrophied intramuscular elastic network, emptied of its muscle cells In other instances the interruption of the continuity of the muscle coat is filled by a scar which is notable because of its high content of elastic fibrils (fig 2 B) Purely collagenous scars are also seen in such locations

The adventitial increase of elastica appears in the connective tissue sheath which surrounds the exterior surface of the muscle coat and is quite distinct from the loose connective tissue of the subserosa It consists of elastic bundles made out of parallel wavy fibrils coursing along the external limit of the muscular coat and often connected with it These fascicles often straddle the large vessels or the branches of the fundamental nerve plexus In high grade increase the greater part of the adventitia may be occupied by a new-formed elastic layer of remarkable thickness (fig. 1)

Such is the pathologic picture of musculoadventitial elastic hyperplasia Being organoid, systematized and located in the very places where the elastic network is normally most abundant, the change has more of the nature of tremendous hypertrophy than of that of a degenerative lesion

Grading of Systematized Musculoadventitial Increase of Elastica—Nothing is known about the range of variation in the elastic tissue of the normal gallbladder



Fig 2—*A*, section of the wall of an obstructed gallbladder, with atrophied mucosa but still preserved muscle coat, Weigert's resorcin-fuchsin, hemalum, $\times 70$. A dense, lamellated, wavy band of hyaline collagen runs in the perimuscular tissue layer. Numerous transitional figures show that this is the last stage of hyaline transformation of an adventitial elastic layer.

B, section of the wall of a gallbladder, Weigert's resorcin-fuchsin, hemalum, phloxine, saffron, $\times 40$. In the center, under a localized thinning of the mucosa, the hypertrophied muscle coat is the seat of a narrow vertical scar, made almost entirely of parallel elastic fibrils. A few muscle bundles still bridge the gap in the media. There are a few interfibrillar elastic deposits in the tunica muscularis. In the perimuscular connective tissue layer one sees a thick new-formed band of elastic fibers. Thickened arteries and small amputation neuromas are also visible in the same layer.

However, it seems safe to assume that elastic deposits visible at a magnification of $\times 60$ are pathologic. At that magnification 55.4 per cent of surgical specimens show no trace of parietal elastica, outside the vessel walls, while 47.6 per cent exhibit various degrees of increase of elastica. One may distinguish minimal elastogenesis (grade 1), which differs from the preceding only because elastica is seen in a few muscle fascicles. In grade 2 many elastic streaks are seen in the tunica muscularis (97 per cent), while in grade 3 deposits of elastica are seen also in the perimuscular tissue layer (83 per cent). Lastly, cases of grade 4 are those in which, beside more or less developed increase of elastic tissue of the muscular coat, a new-formed adventitial layer is seen, stretching for long distances, in two of three sections (73 per cent).

Histogenesis of Systematized Elastic Overgrowth—According to Chiray, Pavel and Lomon,³ production of elastic fibrils occurs during subacute cholecystitis, in the edematous young connective tissue which is then present in the adventitia of the gallbladder. There, according to their description, fibroblasts arrange themselves in fascicles, and are progressively transformed into smooth muscle cells, while numerous elastic fibrils appear in their interstices. Thus, the old muscle coat is finally doubled externally by a reticulated musculoelastic layer which may attain considerable thickness. No mention is made of elastogenesis in the pre-existing muscle layer.

In the material of the Institute of Pathologic Anatomy of the University of Montreal, elastic deposits are first observed in the tunica media, as is evident in a study of low grade elastica increase in almost normal gallbladders. Even in cases in which the change is still slight, there is no sign of actual or of past inflammation in the muscle bundles. In most cases there is slight or strong hypertrophy of the tunica media. However, the increase may happen without appreciable hypertrophy. Indeed, morphologic analysis shows that progressive interfibrillar deposition of elastica is accompanied first with thinning, then with atrophy and disappearance of smooth muscle cells.

In the adventitial layer the histogenesis of elastic fibrils is more difficult to elucidate. There is no parallelism between the increase of elastica and the degree of fibrous postinflammatory thickening of the perimuscular layer. Part of the elastic bundles seem to be left over after the disappearance of aberrant muscle fascicles, since many are continuous with oblique muscle tracts connected with the media. Others straddle the big vessels and nerves of the adventitia, far from the media, and so are in locations where muscle bundles are not found. They thus seem to be formed directly from connective tissue, without previous production of smooth muscle.

Fate of Elastic Tissue—Two possible fates of new-formed elastica may be observed. One is secondary to destructive changes with dense scarring of the wall, and consists in degeneration. It is often seen at the neck of the gallbladder in chronic ulceration due to impaction of a gallstone. The other is progressive disappearance of elastin, which is replaced by fibrohyaline tissue. Such hyaline metamorphosis is seen in the adventitia in some cases of hydrocholecystis and appears as a dense lamellated wavy band of collagen in the adventitia (fig 2 A).

Special Cases and Associated Lesions—In the series of positive cases on which this article is based, biliary lithiasis was constant, with one exception. In 1 case of papilloma of the gallbladder, increase of elastic tissue, grade 1, was found in a nonhypertrophied muscle coat. In strawberry gallbladders the incidence of new elastic fibers was remarkably low (17 per cent). In high grade increase three wall changes were remarkably frequent, being seen in about half the specimens.

The first was hyperplastic endarteritis, the second, disruption of the muscular coat with development of peculiar transverse elastic scars, as described in a foregoing section, the third was hypertrophy of the nerve plexus in the middle and outer layers, with an occasional amputation neuroma (fig 3) On the other hand, hyperplasia of the mucosal nerve plexus, a lesion often associated with cholesterosis, was not particularly frequent

I have observed one surgical and two postmortem specimens of distended gallbladder with stone impaction of the common bile duct and retention jaundice In those specimens, which were exceptions to Courvoisier's law, there was well developed musculo-adventitial overgrowth of elastic tissue In 1 case of enormous distention of the viscus due to pancreatic carcinoma, there was no increase of elastica, but numerous purely fibrous scars were observed in the muscular coat One finds increase of elastic fibers in a high percentage of cases of hydrocholecystis In old cases the wall tends to become entirely fibrous In intermediate stages one may see progressive disappearance of adventitial elastica

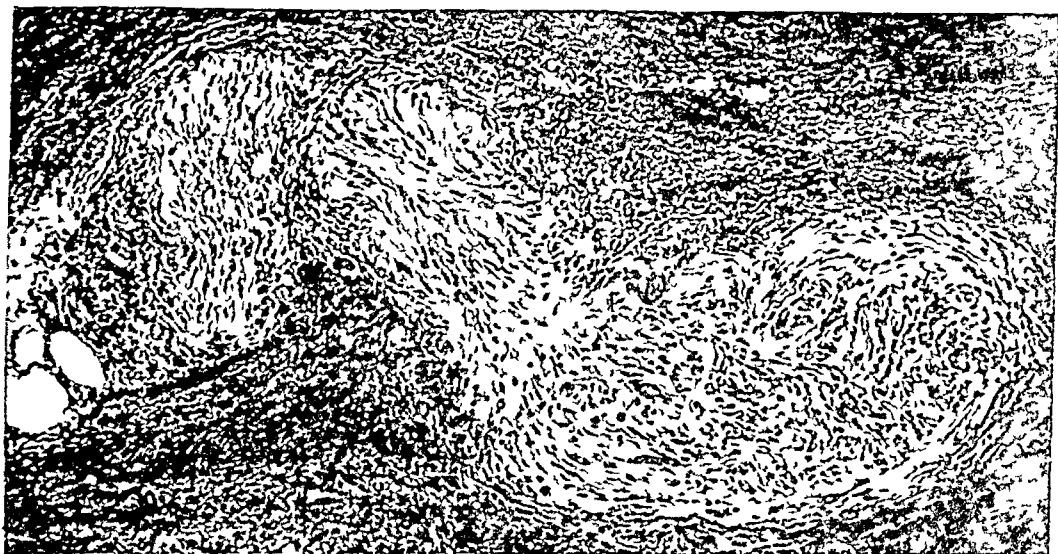


Fig 3—Amputation neuroma of the adventitial layer, together with elastic fibers, Weigert's resorcin-fuchsin, hemalum, phloxine, saffron, $\times 100$ Amputation neuromas and hypertrophy of the fundamental nerve plexus are frequent in gallbladders which show high grade increase of elastic fibers and rare in those which show no appreciable overgrowth of elastic fibers

through hyaline metamorphosis (figs 1B and 2A) In 2 cases of mucocele without distention there was no apparent change in the elastic network

Gross Pathologic Aspects—There is no characteristic gross picture of fully developed musculo-adventitial elastic overgrowth The mean length of 54 gallbladders in which increase of elastica occurred was 82 cm, with variation from 5 to 18 cm—that is, nearly the same as the mean length of the controls (80 cm with variation from 4 to 12 cm) The wall is slightly thickened and hardened In a former work, the mean thickness of the walls of 30 gallbladders in which increase of elastica occurred was 2.6 mm, with extremes of 1.25 and 4 mm, measured on histologic sections As mentioned in a foregoing section, lithiasis is practically constant In most cases the bile is more or less diluted Rarely it is normally colored or colorless

CLINICAL AND ROENTGNOLOGIC DATA

The sex distribution of grade 4 elastic overgrowth differs from that of the control series, as the ratio of males to females is 1 to 1825 in the former and 1 to 675 in the latter, a discrepancy which is not easily ascribed to random sampling⁵

The hypothesis that the ratio 1/675 is preserved may be rejected at a level of 8 per cent

In the present series the mean age of patients with grade 4 increase of elastic fibers is 50.67 years at operation, whereas that of persons without increase is 44.68 years, a statistically significant difference⁶ Strawberry gallbladders without increase of elastic fibers seem to be removed a little earlier (mean age of 61 patients, 41.77, which is significant at the 6 per cent level⁷)

The symptoms are not characteristic. However, the general impression is that in the present series of cases as compared with other cases of disease of the gallbladder pain is less dominant, at least in the last stages, the chief complaint being reflex digestive disturbances. More interesting is the study of the mean duration of the clinical history, which is decidedly longer in cases of grade 4. In these cases the mean duration of symptoms is 7.5 years, compared with 5 years in cases without increase of elastica. This difference is statistically significant⁸

Operation reveals dilatation of the gallbladder in half the cases, and the viscus is described in surgical protocols as big, large, tall, long, dilated, distended, enormous. In only 2 instances was it said to be small, scleroatrophic. In all cases

5

	Males	Females	Total Number
fo	4	69	73
fth	9	64	73

$$\chi^2 = \sum \frac{(f_o - f_{th})^2}{f_{th}} = \frac{25}{9} + \frac{25}{64} = 3.2$$

$$\chi^2 = 3.2 \quad df = 1$$

6

Elastic Increase	Patients	Mean Age	α
Grade 4	73	50.67	11.823
No increase	229	44.69	12.14

$$D = 50.67 - 44.69 = 5.98, \quad \alpha_D = 1.43, \quad \frac{D}{\alpha_D} = \frac{5.98}{1.43} = 4.18$$

7

	Patients	Mean Age	α
No increase of elastica	229	44.69	12.14
Cholesterosis	61	41.77	12.67

$$D = 44.69 - 41.77 = 2.92, \quad \alpha_D = 1.846, \quad \frac{D}{\alpha_D} = \frac{2.92}{1.846} = 1.59$$

8

	Patients	Mean Duration of Symptoms	α
Increase of elastica, grade 4	45	7.5	4.75
No increase	82	5.13	4.74

$$D = 7.5 - 5.13 = 2.37, \quad \alpha_D = 0.716, \quad \frac{D}{\alpha_D} = \frac{2.37}{0.716} = 3.3$$

NOTE α is large relative to the means, indicating that the means may not be very reliable

save 1 there were calculi in the extrahepatic biliary tract, that is, they were nearly always in the gallbladder, in one fifth of the cases they were in the cystic duct, and in one tenth of the cases, in the common bile duct. Adhesions around the gallbladder are mentioned in one third of the cases.

Roentgenologic examination reveals group differences between cases with and cases without increase of elastica, even if restricted to the study of the degree to which the radiopaque dye is concentrated in the gallbladder. For the sake of comparison, all cholecystograms tabulated here were done at Hôtel-Dieu de Montreal by Dr Albert Jutras according to his own technic.⁹ Dr Jutras' technic may be briefly described as cholecystography with the patient in upright position and with graduated compression of the gallbladder after two peroral doses of iodoalphonic acid N N R (priodax® [β -a-(4-hydroxy-3, 5-diodophenyl)-alpha-phenylpropionic acid] or dikol® [same chemical composition as priodax®]). The results are shown in table 1.

TABLE 1—*Roentgenographic Visualization of Gallbladder in Cases with Increase and Cases Without Increase of Elastica*

Cases	Visualization by Cholecystography		
	Normal	Weak	None
No increase in elastica (55 cases)	13 (23.6%)	29 (52.8%)	13 (23.6%)
Increase in elastica, grades 1, 2 and 3 (43 cases)	4 (9.3%)	20 (46.5%)	19 (44.2%)
Increase in elastica, grade 4 (40 cases)	0 (0.0%)	15 (37.5%)	25 (62.5%)

In another series of 56 consecutive cases in which the gallbladder was normally opaque and was removed for calculi soon after cholecystography, the proportion of cases with and cases without increase of elastica was as shown in table 2.

TABLE 2—*Incidence of Increase of Elastica in Cases in Which Gallbladder was Normally Opaque Roentgenographically and Was Removed Because of Calculi*

	No Increase	Increase	
		Grades 1, 2 and 3	Grade 4
56 cases	48 (85.7%)	8 (14.3%)	0 (0.0%)

If there were no relation between increase of elastic tissue and cholecystographic results, the frequency of grades 1, 2 and 3 should be about 40 per cent in the last series, that is, nearly thrice the observed figure. This is further proof of the relative incompatibility of increase of elastic fibers and good roentgenographic visualization.

Filling and emptying time have not been compared up to now in visualized specimens.

PATHOGENESIS OF THE HYPERPLASIA OF ELASTIC TISSUE

The causation of the increase that occurs in the elastica of the diseased gallbladder is still a matter of conjecture, and its complete elucidation would probably require the help of experimentation. How-

⁹ Jutras, A. J. de l'Hôtel-Dieu de Montreal 15 227, 1945.

ever, what is known of the lesion and its etiologic factors justifies a brief discussion of the pathogenesis

Four different possibilities may be considered in regard to the kind of process which may condition the lesion, namely, degeneration, inflammation, postinflammatory regeneration and reaction to mechanical disturbances

The first possibility, degeneration, is suggested by the fact that patients with high increase of elastic tissue have a higher mean age at operation, a fact which may point to a certain analogy with senile elastosis of the skin. In the present cases, however, the elastic deposits show an organoid structure which is entirely at variance with the ordinary concept of degeneration and which is completely missing in senile skin. Moreover, the deposits consist of elastin instead of elacin or collastin, as can be shown by differential stains. Typical cases may occur at an early age, and all cases are distributed rather evenly in frequency distribution curves, without undue frequency in old age. In those patients, the higher mean age at operation signifies only that increase of elastica requires some interval of time, and, consequently, that operation has been delayed.

The second possibility is that new formation of elastica is due to inflammation, especially to inflammation of protracted type. That opinion is based on the observation that there is coincidence of increase of elastic tissue and inflammatory changes in the wall of the gallbladder and that there is seeming formation of elastic fibrils in subacute reactions of the adventitial layer. Against that, one may object that the earliest formation of elastica is observed between muscle fibers, without any evidence of inflammation of the tunica muscularis. Coincidence is inevitable because of the frequency with which inflammatory changes occur in diseased gallbladders. These changes or their sequels are neither constantly present with, nor parallel in intensity to, the increase of elastic tissue, as may be seen in fibroplastic pericholecystitis. Lastly, general pathology teaches that, far from being a stimulant to elastogenesis, inflammation per se has destructive effects on elastic networks.

A regenerative, postinflammatory origin is a better founded possibility. It is true that usually scar tissue is almost devoid of elastic fibrils and that regeneration of elastic networks is very late in cicatrices. However, it is a curious property of the fibrous tissue seen in cirrhosis of the liver as well as in scars of kidneys, that it may show conspicuous formation of elastic fibrils (Hass¹⁰). The connective tissue of the gallbladder may share with that of the liver such a peculiar property

That possibility cannot be ruled out as far as the diffuse formation of elastic fibrils seen in undifferentiated connective tissue of the gallbladder is concerned. However, it seems inadequate in explaining the location and the character of the muscularis and adventitial increase of elastic fibrils and in explaining other, accessory facts, as appears in the last part of the discussion.

The last possibility, that of a mechanical origin, is based on many concurrent data. These are the tendency toward distention of the gallbladder, the frequency with which calculi are found impacted in the cystic duct, the high incidence of defective roentgenologic visualization in spite of the absence of true cholecystitis, the great variation of content of biliary pigment, all of which point to stagnation of the bile of the gallbladder. The frequent disruptions of the muscle coat and of the deep nerve plexus suggest, also, the possibility of insufficiency and incoordination of muscle contraction. Lastly, the very nature and character of the new-formed substance and its relationship to muscle fibers point to a mechanical determinism in its deposition in the gallbladder wall.

This mechanical condition is probably not a purely continuous stress, that is, simple stretching of the wall, which leads to fibrous atrophy with disappearance of elastin, as is seen in old hydrocholecystis. According to all indications, it requires intermittent dilatation, and ceases after complete anatomic exclusion.

Formal genesis could thus be resumed as a loss of motor control of the gallbladder over its contents and volume, before complete anatomic exclusion of the organ, with intermittent, predominantly passive, volumetric changes, the motor insufficiency being secondary or even primary.

In most cases motor deficiency seems to follow a phase of overwork, which is evidenced by the discovery of a mechanical obstacle, on one side, and by the existence of muscle hypertrophy, on the other. It may then be called secondary motor deficiency. In other cases there is a suggestion of diminution in the power of contraction without sign of previous overwork (primary deficiency). In most instances scars may account for such primary deficiency. In rare cases there is no scarring, no disruption of nerves, no evidence of previous hypertrophy. The muscle is atrophic and partially replaced by elastic tissue. A defective or insufficient stimulation of the muscle coat may then be invoked as a cause of primary insufficiency.

Be that as it may, increase of elastic tissue deserves to take its place among changes which are probably indicative of mechanical perturbation of the function of the gallbladder with stagnation of bile. Its presence may result in preventing excessive dilatation of a permanent and irreversible nature, while the "asystolic" gallbladder is still

connected with the biliary tract. It could then be considered as an adaptive mechanism coming into play when there is a modification between the forces which govern the motions of the gallbladder.

If such is the case, considering the frequency of the lesion and the relative incompatibility of even slight increase of elastica with good roentgenologic visualization of the viscus, one may surmise that mechanical troubles play a larger role, and defective concentration, a lesser one, than formerly thought, in defects of gallbladder visualization.

SUMMARY

In the normal state the wall of the gallbladder is poor in elastic fibers, a fact which may explain the neglect of pathologists in assessing the morbid variations of these components. However, disease of the gallbladder is often attended with slight or pronounced increases of parietal elastica.

The present work describes the pathologic aspects of this neglected lesion, its frequency according to grades, and its histogenesis, as well as the associated alterations of the wall of the gallbladder.

The mean age at operation of patients with high grade increase of elastica is statistically higher than that of patients without such increase. The clinical duration of the disease is likewise significantly longer in the former. There are group differences in the roentgenographic opacity of the specimens between cases without and cases with elastic increase.

The most probable hypothesis concerning the pathogenesis is that there is mechanical trouble in the filling-emptying cycle of the viscus.

PATHOGENESIS OF EXPERIMENTAL HEPATIC FIBROSIS AND CIRRHOSIS IN THE DOG

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IN THE laboratory of the division of physiology of the University of California Medical School, a study has been made of the reaction of the dog's liver to the following procedures: pancreatectomy¹, the feeding of a high fat, low protein diet², excision of the thyroid and/or the pituitary gland³. The collection of these cirrhotic livers during the past has provided a unique opportunity for critically comparing the different forms of cirrhosis that can be produced in the dog and for comparing these reactions with other experimentally induced hepatic lesions described in the literature⁴. Through this comparison it has been possible, for the first time, to distinguish and define clearly the genesis of several forms of fibrosis in the dog and to establish the relation between the initial reactions and the cirrhosis which ultimately supervenes. This comparative study has provided some insight, first, into the significance of hepatocellular damage and circulatory changes in the initiation of hepatic fibrosis and, second, into the relation

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1 Chaikoff, I L, Connor, C L, and Biskind, G R. *Am J Path* **14** 101, 1938

2 Chaikoff, I L, Eichorn, K B, Connor, C L, and Entenman, C. *Am J Path* **19** 9, 1943

3 Chaikoff, I L, Gillman, T, Entenman, C, Rinehart, J F, and Reichert, F L. *J Exper Med* **88** 373, 1948

4 (a) Moon, V H. *Arch Path* **18** 381, 1934 (b) Gyorgy, P. *Am J Clin Path* **14** 67, 1944 (c) Glynn, L E. *Nutrition Abstr & Rev* **16** 751, 1946 (d) Glynn, L E, Himsworth, H P, and Lindan, O. *Brit J Exper Path* **29** 1, 1948 (e) Glynn, L E, and Himsworth, H P. *J Path & Bact* **56** 297, 1944 (f) Zimmerman, H M, and Hillsman, J A. *Arch Path* **9** 1154, 1930 (g) Mallory, F B, Parker, J, Jr, and Nye, R N. *J M Research* **42** 461, 1920-1921 (h) Chaikoff, I L, Entenman, C, Gillman, T, and Reichert, F L. *Proc Soc Exper Biol & Med* **67** 345, 1948 (i) Chaikoff and others, footnotes 1, 2 and 3

between the etiologic factors and the form of cirrhosis which is detected at autopsy

NOMENCLATURE

At the outset it is necessary to define the terms to be used here. The term "hepatic fibrosis" will be applied to a histologically detectable increase of the connective tissue of the liver. When all states have been observed in the same liver between the earliest detectable increase of connective tissue and frank cirrhosis, the lesion will be referred to as precirrhosis. "Cirrhosis" will be used to describe a marked increase of hepatic connective tissue associated with obvious distortion of the lobular structure, particularly a derangement of the usual relationship between neighboring lobules, or fibrous tissue disfigurement of large numbers of individual hepatic lobules.

Terms like "interstitial fibrosis," "interlobular fibrosis" and "intra-lobular fibrosis" are so widely used and correctly interpreted in pathology as to require no special mention here.

TYPES AND GENESIS OF HEPATIC FIBROSIS AND CIRRHOSIS ENCOUNTERED IN THE DOG

The forms of fibrosis seen in the dog may be classified into three main groups

- 1 Centrilobular or peri-hepatic vein fibrosis
- 2 Periportal fibrosis
- 3 Diffuse interstitial or intralobular fibrosis

As will appear from the following descriptions, these names refer to the location in which the excess fibrous tissue proliferation is initiated, for, with the progress of a lesion, one form may become widespread and develop into, or become associated with, a second or a third form. For example, a centrilobular fibrosis may, by extending into the tissue more widely removed from the radicles of the hepatic vein, develop into diffuse interstitial fibrosis. On the other hand, a lesion apparently commencing interstitially some distance from the central vein may later spread to the central region and become, for a time, localized there.

In the very late stages of cirrhosis, when structural distortion is gross, it may be impossible to determine in any single section the mode of development of a particular cirrhotic lesion. However, we have repeatedly found, even in the same section or in sections from different lobes of the same liver, several stages in the evolution of a particular form of cirrhosis.

- 1 *Centrilobular or Peri-Hepatic Vein Fibrosis* (figs 1 to 10) — Before 1946 this form of hepatic fibrosis had been described only in

patients with chronic cardiac decompensation⁵ and in dogs with surgically impaired hepatic venous return^{4f}. However, the recent observations of Lillie and associates⁶ were subsequently extended, Ashburn, Endicott, Daft and Lillie,⁷ through a series of careful experiments, demonstrated conclusively that in the rat several forms of experimentally induced hepatic fibrosis previously regarded as periportal in origin actually developed around the radicles of the hepatic veins.

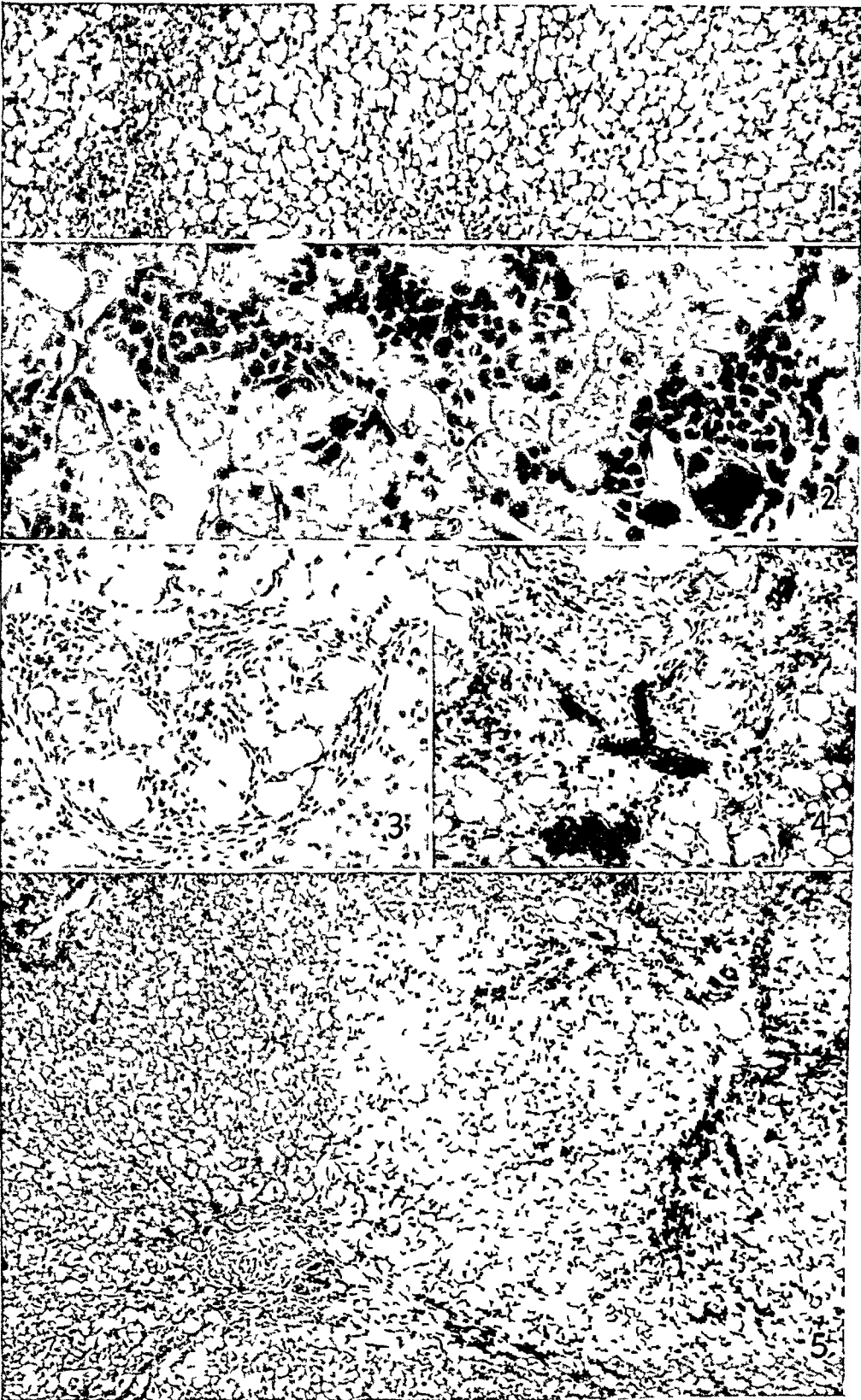
In our series of dogs, centrolobular or peri-hepatic vein fibrosis occurred in animals subjected to a variety of experimental procedures, including pancreatectomy, the administration of large quantities of alcohol to animals maintained on a high fat or a high protein diet, and ablation of both the thyroid and the pituitary gland or of the thyroid gland alone.

In the majority of dogs this form of fibrosis was encountered in livers showing various degrees of fatty change. The fatty change was usually most marked toward the centers of the lobules (fig 1), and it was around the enlarged, very fatty liver cells that the initial changes were usually detected. These early reactions consisted of edema of the small rim of connective tissue and reticulum supporting the central vein, together with a collection of cells, including some polymorphonuclear leukocytes and one or more giant cells (fig 2). Such cellular infiltration was not regularly encountered in all the affected lobules. In some livers the earliest change was edema and increased eosinophilia or basophilia of the reticular fibers between and around the enlarged, fatty liver cells near the central zone (figs 3, 4 and 5). This change in the pericellular and intercellular reticulum, detected in routine hematoxylin and eosin preparations, was shown (by special silver methods for demonstrating the reticular fibers) to represent a distinct thickening of the individual argyrophilic fibers, which soon became more numerous, although they occupied the usual intercellular positions. Such thickening and increase of the amount of reticular fibers have been described in human cardiac cirrhosis by several investigators,⁵ in dogs^{4f} and in nutritional cirrhosis of rats⁶. In our dogs this change of the reticulum was soon succeeded by an actual increase of fibrocytic and fibroblastic cells as well as of monocytes, lymphocytes and thrombocytes, which still occupied intercellular and pericellular positions along the line of the altered reticulum.

5 Lambert, R. A., and Allison, B. R. *Bull. Johns Hopkins Hosp.* **27**: 350, 1916. Katzin, H. M., Waller, J. V., and Blumgart, H. L. *Arch. Int. Med.* **64**: 457, 1939. Koletsky, S., and Barnebee, J. H. *Am. J. M. Sc.* **207**: 421, 1944. Costero, I., and Barroso-Miguel, R. *Arch. Inst. Cardiol. Mexico* **17**: 337, 1947.

6 Lillie, R. D., Ashburn, L. L., Sebrell, W. H., Daft, F. S., and Lowry, J. V. *Pub. Health Rep.* **57**: 502, 1942.

7 Ashburn, L. L., Endicott, K. M., Daft, F. S., and Lillie, R. D. *Am. J. Path.* **23**: 159, 1947.



Figures 1-5
(See legends on opposite page)

This reaction may remain localized and progress extremely slowly, in which case an intralobular fibroma-like lesion may occur. In such instances the enlarged, fatty liver cells remain intact and apparently healthy, although each cell is surrounded by young fibrous tissue (fig 3). Even the focus of this reaction may be totally encapsulated by a thin rim of fibrous tissue (fig 3). Within such a fibroid-like lesion, giant cells with single multilobular and contorted nuclei are occasionally encountered. Such lesions, occurring in fatty tissue, have been previously described, so far as we know, only in the subcutaneous fat of the extremities⁸. In the latter site this reaction is succeeded by fibrosis and atrophy of the subcutaneous fat, with consequent puckering of the overlying skin.

In the liver such localization of the initial fibrous tissue reaction is unusual, for as a rule the fibrosis is more widespread and extends fairly rapidly into the adjacent centrilobular fatty liver tissue. In figure 5 the lesion has apparently commenced slowly and remained localized at the outset, as in figure 3, but the reaction has then spread quite extensively among the adjacent fatty parenchymal cells. Figure 4 depicts an intermediary rate of development, in which the fatty cells

8 Gilchrist, T. C., and Ketron, L. W. *Bull. Johns Hopkins Hosp.* **27**: 291, 1916.

EXPLANATION OF FIGS 1-5

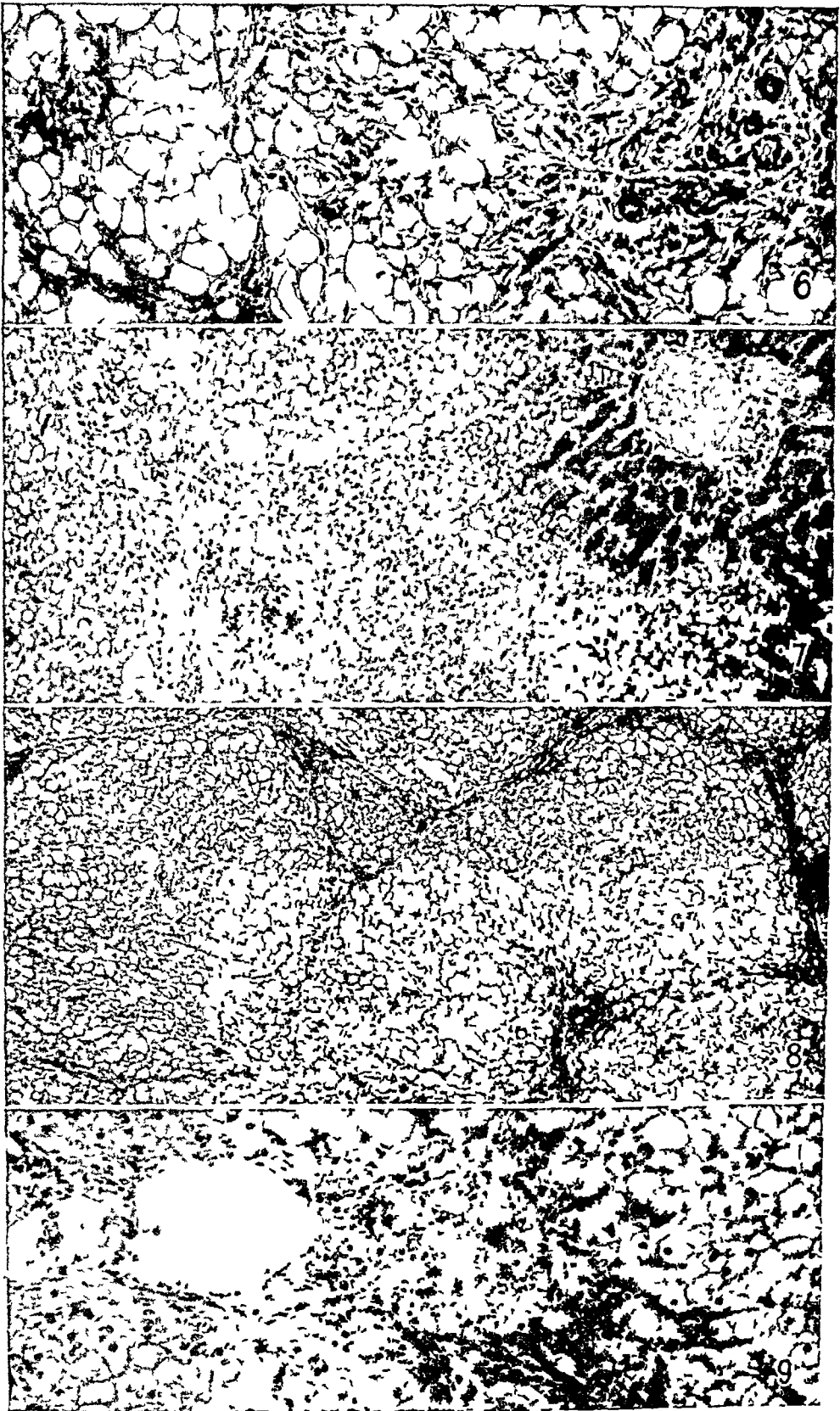
Fig 1 (dog D28, depancreatized 15 weeks prior to microscopic examination of liver, maintained with insulin and a lean meat diet).—The fatty change is most marked toward the center of the lobule, while the rim of cells toward the portal tracts is virtually free of fat. The central vein is at bottom center. Hematoxylin and eosin, $\times 115$.

Fig 2 (dog DE, depancreatized 23 years prior to examination, maintained with insulin and a lean meat diet).—Focal accumulations of cells, among which are a few polymorphonuclear leukocytes and occasional mononuclear giant cells, near the longitudinally sectioned central veins on the left, such as depicted here, are typical of the early reactions which may be followed by fibrosis. Hematoxylin and eosin, $\times 340$.

Fig 3 (dog D66, depancreatized 36 weeks prior to examination, maintained with insulin and a lean meat diet for first 20 weeks, raw pancreas added to diet for last 16 weeks).—The fibrosis around the centrilobular fatty liver cells is fairly well advanced, although localized and encapsulated by fibrous tissue. Hematoxylin and eosin, $\times 140$.

Fig 4 (dog F10, high fat diet plus alcohol for 4 months).—Several stages in the course of centrilobular fibrosis are shown. The central mass of fibrous tissue, with new capillaries, represents a fairly advanced stage of a comparatively localized fibrosis. Around this central mass, and especially toward its lower right corner, can be seen the earlier stages of intercellular edema and thickening of the reticulum, with increased cellularity between the fatty liver cells. Hematoxylin and eosin, $\times 125$.

Fig 5 (dog HT8, hypophysectomized and thyroidectomized 419 days prior to examination).—Toward the bottom is shown a localized fibroid-like change among the fatty liver cells, with the fibrosis extending in all directions among the fatty cells. An early associated periportal fibrosis is also visible (upper right corner). Hematoxylin and eosin, $\times 70$.



Figures 6-9
(See legends on opposite page)

enclosed within the initially affected area have become completely obliterated by the fibrotic process (compare fig 3 with intact fatty liver cells) In the latter instance, capillaries have appeared within the focus of fibrosis Moreover, the earlier stages of the fibrotic process have also made their appearance around the fatty cells immediately abutting on the almost rectangular central area of fibrosis

When centrolobular fibrosis is acute and highly active, it adopts the form depicted in figure 6 In these instances the alterations of the reticular fibers described in a foregoing paragraph spread rapidly and erratically in all directions throughout the lobule and may soon involve the portal tracts When the centrolobular fibrosis spreads thus rapidly, it may become difficult to distinguish its site of origin, and consequently such a lesion may be taken to be interstitial fibrosis However, the character of the new connective tissue in the centrolobular lesion is quite different from that encountered in the forms of interstitial fibrosis described in a later section of this paper (compare figs 5, 6 and 8 with figs 16, 17 and 18)

On several occasions we have seen in depancreatized dogs and in animals fed a high fat diet with or without alcohol an acute hemorrhagic necrosis supervening in the centrolobular fatty parenchyma Such a hemorrhagic change may be widespread throughout the liver or may be confined to one or more lobes Should the animal survive, the hemorrhage is followed by fibrosis within the centrolobular necrosis resulting from the hemorrhage (fig 7) In such instances the reaction

EXPLANATION OF FIGS 6-9

Fig 6 (dog D24, depancreatized 39 weeks prior to microscopic examination of liver, maintained with insulin and a lean meat diet for first 33.5 weeks, raw pancreas added to diet for last 5.5 weeks) —The fibrosis around the fatty liver cells is highly active and progressing rapidly There is an associated early periportal fibrosis Hematoxylin and eosin, $\times 130$

Fig 7 (dog F57, fed a high protein diet plus alcohol for 9 weeks) —In this specimen a centrolobular hemorrhage has occurred, with incipient fibrosis, among the centrolobular fatty liver cells Note that the periportal areas show no abnormal reaction Hematoxylin and eosin, $\times 92$

Fig 8 (dog HT8, hypophysectomized and thyroidectomized approximately 36 weeks prior to examination) —Fibrosis localized within the area of fatty change is clearly shown The fibrous tissue in the centrolobular regions of adjacent lobules has now joined to form a fibrous ring enclosing portions of several lobules around centrally located, small portal tracts Several such fibrous rings portrayed in this picture give the appearance of a monolobular cirrhosis There is some extension of the fibrosis among the less fatty neighboring liver cells, together with some reaction in one of the portal tracts lying centrally within a fibrous ring Hematoxylin and eosin, $\times 70$

Fig 9 (dog D12, depancreatized 34.5 weeks prior to examination, maintained with insulin and a lean meat diet for 28.5 weeks, raw pancreas added to diet for last 6 weeks) —A branch of a hepatic vein with eosinophilia of the connective tissue and associated fibrosis spreading among the surrounding degenerating liver cells This peculiar fibrotic change is not obviously related to the fat in the liver cells Hematoxylin and eosin, $\times 170$

resembles closely the more acute changes observed by Zimmerman and Hillsman ⁴¹ in dogs with surgically induced obstruction of the inferior vena cava. Although in these instances the fibrosis is precipitated acutely after the hemorrhage, the lesion subsequently progresses in a manner almost identical with that described in foregoing paragraphs as occurring in the fatty liver.

Figure 8 shows what happens when the liver is involved in a centrolobular fatty change and fibrosis. Here it can be seen that in each lobule the centrally lying fat has become affected by a fibrotic process which has spread along the radicles of the hepatic veins. Frequently the central vein is not detectable, because of the intensity of the fatty change, and it becomes clear that the larger ramus of the hepatic veins are similarly affected by perivascular fat and fibrous tissue changes. Moreover, the fibrosis around the hepatic radicles causes adjacent lobules to join one another, so that rings of fibrous tissue formed through this union of the central zones of three or more adjacent lobules can easily be recognized, the center of the fibrous ring or circle being represented by a portal tract. Superficial examination gives the impression that this is a monolobular cirrhosis, but in actual fact several neighboring lobules are participating in the process (fig 8).

When the aforementioned lesions progress gradually, their centrolobular or peri-hepatic vein origin can be distinguished without difficulty. However, in the subsequent stages the fibrous tissue rings contract, the fibrosis extends and the portal tracts are drawn into the zone of fibrosis. The centrolobular lesions may often be complicated with the supervention of both interstitial and periportal fibrosis. In such instances the distortion of hepatic structure is not only more impressive but more precipitous, frequently making the distinction between centrolobular and periportal cirrhosis difficult, and can be made out with certainty only by studying the genesis of the lesion in different parts of the same section.

One other fibrotic reaction around the radicles of the hepatic vein has been encountered in dog livers. In this reaction a peculiar form of fibrosis is initiated in the connective tissue which supports the hepatic vein itself. The hepatic cells in this region appear to undergo eosinophilic degeneration, which makes them denser and more obvious. This degeneration is succeeded by an increase of fibrocytes, and later by a highly eosinophilic form of dense connective tissue which can be seen insinuating itself into the surrounding parenchyma (fig 9). While this lesion usually occurs in a mildly fatty liver, the connective tissue reaction cannot be related in any obvious way to the fatty change. The progress of this form of peri-hepatic vein fibrosis culminates in cirrhosis in the manner described in the foregoing paragraph.

2 *Periportal Fibrosis and Cirrhosis* (figs 10 to 15) —In its most widely recognized form this fibrotic change in the liver commences with an accumulation of polymorphonuclear leukocytes and other cells in the portal tracts together with edema of the periportal collagen fibers. As has been demonstrated previously, in hypophysectomized and thyroidectomized dogs,⁹ periportal fibrosis may, in its initial stages, closely resemble the early stages of the cholangiolitic type of cirrhosis observed in human subjects by Watson and Hoffbauer⁹ and regarded by them as secondary to chronic or repeated attacks of hepatitis. It is unlikely, however, that an infective hepatitis could have been the cause in our dogs. Following the cellular infiltration there is an increase in the amount of collagen in and around the smaller portal tracts, and when this connective tissue spreads into the adjoining parenchyma and distorts the lobular pattern, cirrhosis supervenes. A similar process has also been seen in depancreatized dogs. In these animals the fibrosis usually occurs in a liver showing predominantly periportal fatty changes (fig 10). However, in the later stages the relationship between the periportal location of the fat and the advanced cirrhosis is not detectable, since the fatty change affects the liver more diffusely (fig 11). Thus, while the liver depicted in figure 10 contained 14 per cent total fatty acids, that in figure 11 contained 29 per cent total fatty acids.

In the later stages, too, hyperplasia of bile ducts is commonly seen in the densely fibrotic portal zones (fig 11). Unless fibrosis commences elsewhere in the parenchyma, i. e., interstitially or centrolobularly, the disease progresses slowly, and structural distortion is long delayed. Such a slowly progressive portal fibrosis may result in a monolobular-like cirrhosis (fig 12) closely resembling that following slow centrolobular fibrosis (compare fig 12 with fig 8). Whereas in figure 12 the central veins are obviously unaffected by the fibrosis and lie in the usual position, in figure 8 the central veins of adjacent lobules have become joined by fibrous tissue which, forming a ring, surrounds the nonfibrotic, centrally located portal tracts. Superficially these two processes resemble one another closely, but careful study reveals their fundamental differences. However, in depancreatized dogs and in normal animals fed a high fat diet the type of interstitial fibrosis described in the following section commonly occurs in association with the periportal changes, and consequently gross structural distortion rapidly follows the combined effects of periportal and interstitial fibrosis.

In depancreatized dogs, still another form of periportal fibrosis may occur in a relatively nonfatty liver. Thus, in figures 13 and 14, which reproduce sections from a liver containing only 10 per cent total

⁹ Watson, C. J., and Hoffbauer, F. W. *Ann Int Med* 25 195, 1946



Figures 10-13
(See legend on opposite page)

fatty acids, a striking collection of cells, including polymorphonuclear leukocytes and fibroblasts, can be seen surrounding the portal tracts. This type of periportal fibrosis appears to progress rapidly, resulting in an early uniting, by young fibrous tissue, of the neighboring portal areas (fig 14). Interstitial and centrolobular fibrosis usually supervenes early and results in rapid distortion or cirrhosis of these nonfatty livers.

The periportal fibrosis may also begin in the manner described for the peculiar pericentral fibrosis depicted in figure 9, that is, the connective tissue around the portal tracts and adjacent parenchyma may undergo thickening and become intensely eosinophilic (fig 15). With the radial spread of these peculiar changes, fibrosis becomes more extensive and culminates ultimately in cirrhosis. Whereas in figure 9 the reaction is essentially around the hepatic radicles, in figure 15 it is obviously the portal tract which is clearly pathologic.

With periportal fibrosis, as with centrolobular fibrosis, the rate at which gross structural distortion supervenes is determined not so much by the site of inception of the fibrosis as by the extent of the reaction, its speed of progress and the occurrence of fibrosis in other sites. The presence or the absence of lobular hyperplasia will determine to a significant extent the degree of structural distortion and therefore the terminal pathologic picture.

3 *Diffuse Interstitial or Intralobular Fibrosis* (figs 16 to 21) — This type of fibrosis was most frequent in depancreatized dogs and in normal animals restricted to high fat, low protein diets with or without

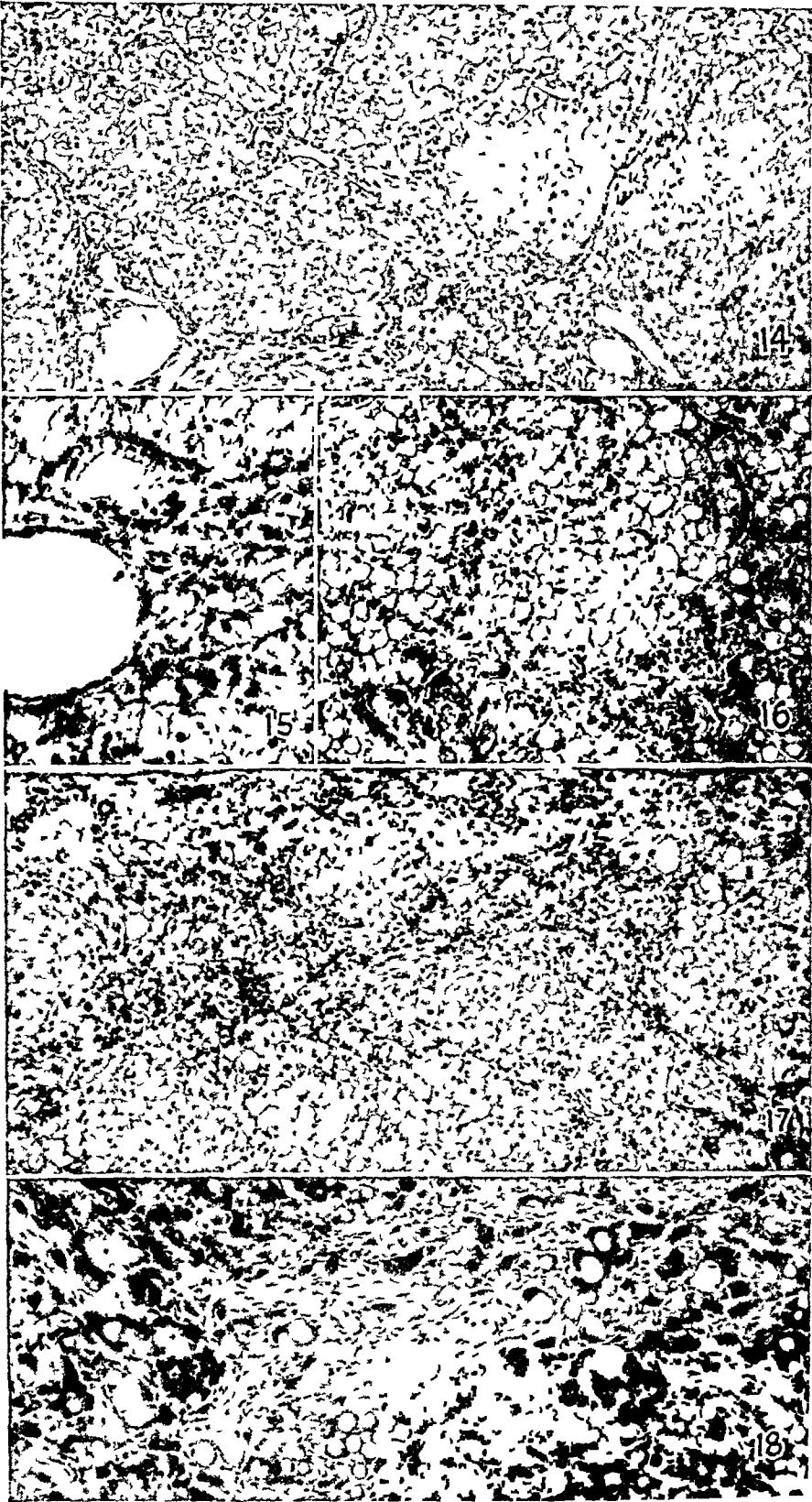
EXPLANATION OF FIGS 10-13

Fig 10 (dog D65, depancreatized 35 weeks prior to microscopic examination of liver, maintained with insulin and a lean meat diet for 20 weeks, autoclaved pancreas added to diet for last 15 weeks) — In this liver the fatty change is most marked among the periportal liver cells. The portal tract is edematous and undergoing early fibrosis, with the fibrous tissue extending along the course of the portal triads immediately related to the most severely affected tract in this picture. There is some associated hyperplasia of bile ducts. Hematoxylin and eosin, $\times 114$.

Fig 11 (dog DG, depancreatized 15 years prior to examination, maintained with insulin and a lean meat diet) — Later stage in portal fibrosis with early structural distortion. Hyperplasia of bile ducts has occurred within the densely fibrosed portal areas. Hematoxylin and eosin, $\times 70$.

Fig 12 (dog DA, depancreatized 55 years prior to examination, maintained with insulin and a lean meat diet) — Late stage in portal fibrosis with bands of fibrous tissue accumulating along the portal tracts and surrounding lobules. The central veins in the affected lobules do not show any significant change. Mallory's phosphotungstic acid-hematoxylin, $\times 70$.

Fig 13 (dog DE, depancreatized 26 years prior to examination, maintained with insulin and a lean meat diet for 23 years, raw pancreas added to diet for last 16 weeks) — A large number of polymorphonuclear leukocytes and early but distinct fibrosis are detectable. This reaction is not obviously related to the comparatively mild fatty change in this liver, nor can biliary obstruction or infection be regarded as the precipitating cause of this lesion. Hematoxylin and eosin, $\times 225$.



Figures 14-18
(See legends on opposite page)

alcohol¹⁰ Although in earlier reports^{10a, c} on the animals subjected to the treatments mentioned "intralobular fibrosis" was referred to, neither its nature nor its mode of development was fully described

The livers affected by such diffuse interstitial fibrosis are almost invariably very fatty In this lesion, numerous small fibroblasts and fibrocytes develop among the enlarged fatty liver cells There does not seem to be any predilection for a particular site in the liver lobule, for, as can be seen from figures 16, 17 and 18, this peculiar fibrosis may commence anywhere in the lobule The portal tracts and central veins are involved rather late

The fibrous tissue may be highly cellular and may spread rapidly through large areas of parenchyma (figs 16 and 17) In such instances the sections of the liver are so cellular as to resemble diffuse hepatic "sarcomatosis" (figs 16 to 20) Entire areas of hepatic epithelium are replaced by whorls of highly cellular connective tissue (figs 19 and 20) In the later stages all that remain of the liver epithelium and bile ducts are scattered small groups of degenerate liver cells and areas of bile duct proliferation (fig 20)

As can be seen from figures 16 to 20, this diffuse fibrosis is not preceded by, or associated with, focal or massive necrosis such as is

10 (a) Conner, C L, and Chaikoff, I L Proc Soc Exper Biol & Med **39** 356, 1939 (b) Chaikoff, I L, Entenman, C, Gillman, T, and Conner, C L Arch Path **45** 435, 1948 (c) Chaikoff and others²

EXPLANATION OF FIGS 14-18

Fig 14 (dog DE, see fig 13) —The periportal location of the cellular infiltration and the early fibrosis in this liver are distinct There is also the early reticular thickening of incipient fibrosis intralobularly These reactions are not related to the fatty change, as the liver is not severely fatty This liver contained only 10 per cent fatty acids Hematoxylin and eosin, $\times 115$

Fig 15 (dog D12, see fig 9) —The connective tissue around the portal tract has undergone a peculiar change with eosinophilia and thickening This reaction also extends into the surrounding parenchyma The hepatic radicle related to this portal tract was apparently normal Hematoxylin and eosin, $\times 170$

Fig 16 (dog K, depancreatized 27 years prior to examination, maintained with insulin and a lean meat diet) —There is diffuse fibrosis throughout the very fatty parenchyma, with liver cells being replaced by fibrous tissue The portal tract toward the lower left corner is only mildly fibrotic in comparison with the severity of the parenchymal changes This liver contained 34 per cent fatty acids Hematoxylin and eosin, $\times 70$

Fig 17 (dog K, see fig 16) —Diffuse interstitial active fibrosis in the subcapsular region The young, cellular connective tissue is apparently spreading in all directions indiscriminately There is early implication of the portal tract at the lower right edge of the picture However, this portal tract shows no cellular infiltration, thickening or edema comparable with that depicted in figures 10, 13 or 14 Hematoxylin and eosin, $\times 70$

Fig 18 (dog F20, fed a high fat diet for 36 weeks) —In the center of the picture the liver cells are replaced by dense bands of collagen fibers which disrupt the epithelial trabeculae On all sides of this band of dense collagen, earlier stages of pericellular fibrosis are shown Hematoxylin and eosin, $\times 112$



Figures 19-21

(See legends on opposite page)

seen in acute infections or toxic hepatitis in man. The impression gained is that some potent stimulus for hyperplasia of connective tissue has been imposed on the hepatic mesenchymal elements, which have responded diffusely and overrun the epithelial parenchyma. In many respects this lesion resembles that described by Karsner¹¹ as "intrahepatic cholangiolitic cirrhosis." But in our dogs, while centrolobular accumulation of bile can be detected as a complication in the late stages, the lesion does not appear to be induced by any detectable form of biliary infection or obstruction. It is possible, however, that some toxin or macromolecule of endogenous or exogenous origin may play a role in the causation of this diffuse hyperplasia of the connective tissue of the liver.¹²

In several livers the process, while progressing actively in some areas (judged by the extent and cellularity of the fibrous tissue), appears to be associated in other parts with the deposition of intralobular bands of eosinophilic collagen. Thus, in figure 18, above and below the center of the picture, active cellular interstitial fibrosis is proceeding, while in the center the early connective tissue is being supplanted by dense bands of collagen winding between and around the liver cells. With the advent of such a process, the hepatic trabeculae become disrupted, and portions of degenerating epithelial cords can be seen ensnared within the spreading connective tissue net (fig 19, center).

Occasionally, the reaction depicted in figure 21 is encountered in livers undergoing interstitial fibrosis. Here chains of elongated, highly eosinophilic cells can be seen stretching indiscriminately through the parenchyma. The nuclei of these cells are small and hyperchromatic.

11 Karsner, H. T. *Am J Clin Path* **13** 569, 1943.

12 Hueper, W. C. *Arch Path* **33** 267, 1942. Gillman, J., Gillman, T., and Gilbert, C. *South African J. M. Sc.*, to be published.

EXPLANATION OF FIGS 19-21

Fig 19 (dog F39, fed a high fat diet for 32 weeks) —The interstitial fibrosis is progressing actively and is well advanced. Highly cellular scars replace patches of hepatic epithelium. The joining of bands of cellular fibrous tissue has resulted in gross structural distortion. Hematoxylin and eosin, $\times 64$.

Fig 20 (dog F39, see fig 19) —In this late stage of extremely active interstitial cirrhosis, a large area of liver parenchyma is replaced by highly cellular connective tissue which looks almost sarcomatous. The liver parenchyma is represented only by isolated groups of islands of fatty liver cells (left of center) and irregular groups of epithelial cells resembling proliferated bile ducts. Hematoxylin and eosin, $\times 130$.

Fig 21 (dog DE, see fig 14) —A peculiar form of highly eosinophilic, cellular connective tissue stretches indiscriminately throughout the parenchyma. This is associated in one area (to left of center) with intrafatty fibrosis. Note also the eosinophilia with nuclear hyperchromasia of the liver cells just below center. Hematoxylin and eosin, $\times 85$.

In some areas (see bottom center of fig 21), the liver cells themselves seem to be undergoing a similar eosinophilic change. However, the nature of the reaction leaves little doubt that, whatever the genesis of this peculiar tissue, the result is diffuse interstitial fibrosis with structural distortion (fig 21). This particular reaction resembles closely that described as occurring either periportal or around the hepatic radicles (compare fig 21 with figs 15 and 9). This reaction appears to be unrelated to the fatty change in the liver cells, although in figure 21 (top, left quadrant) another type of fibrotic change within the fatty areas seems to accompany this other peculiar form of eosinophilic fibrosis.

Structural distortion supervenes early with the progress of this interstitial type of hepatic fibrosis. At no stage during the evolution of this form of fibrosis do the lesions in the liver resemble those portrayed in figures 8, 12 and 14, for the fibrosis does not result in involvement of any localized portion of the lobule, as in the centrolobular and periportal types of fibrosis described. The end picture of this form of fibrosis when it is rapidly progressive is that portrayed in figures 19 and 20.

COMMENT

The four points of significance which emerge from this study of the genesis of hepatic fibrosis and cirrhosis in the dog are the following:

First, in the dog's liver fibrosis may originate periportal, between the parenchymal epithelial cells (interstitial) and/or around the radicles of the hepatic veins (centrolobular). These three forms of fibrosis can usually be distinguished easily in the early stages.

Second, some forms of fibrosis, especially the centrolobular form and perhaps the periportal and interstitial forms, appear to be related to the fatty change. But even in the centrolobular form, in which the association of the fibrosis with fat is usually more obvious, the fatty liver cells may remain quite intact and may not degenerate until the fibrosis is well advanced (fig 3). In fact, the centrolobular fibrosis described here closely simulates, in pathogenesis, the reactions described in chronic cardiac decompensation in man⁵ and in passive venous congestion of the liver in dogs^{4f}. When this reaction occurs in the congested liver, it does not seem to be associated with, or preceded by, fatty changes in the liver cells. Possibly the circulatory changes occurring in a very fatty liver induce effects on the supporting reticulum similar to those occurring after long-standing venous congestion. Should this be the case, then attention must be devoted not only to the hepatocellular degeneration but also to the role of the vascular changes in the liver in the genesis of hepatic fibrosis. Attention has previously

been drawn to this aspect of hepatic fibrosis by Deysach¹³ and later by Glynn and associates^{4d} and by Himsworth and Glynn¹⁴

The phenomena recorded to this point indicate that, as previously stated by Gillman and co-workers,¹⁵ there are factors other than hepatocellular degeneration to be considered as being responsible for proliferation of the connective tissue of the liver. Special conditions seem to be necessary for hyperplasia of the connective tissue of the liver, for fibrosis does not necessarily follow even massive hepatocellular damage¹⁶, in fact, entire lobes of the liver may atrophy and disappear without any associated connective tissue response¹⁵. Not only is this the case for the liver, but a similar observation appears to hold true for the pancreas and the salivary glands¹⁷. Thus, we have encountered reactions primarily in the interlobular septums of the dog's pancreas¹⁸ or interstitial fibrosis in the human pancreas^{15b} or even complete degeneration of the acinous tissue without any connective tissue reactions whatsoever¹⁹.

These observations concerning the liver and the pancreas of man and several laboratory animals indicate that special conditions are necessary for the development of fibrosis in these organs. It would seem that the parenchymal cells and the supporting tissues of these organs, while frequently reacting simultaneously, are nevertheless two independently reacting systems.

The third significant fact emerging from this study is that several different forms of fibrosis can occur in the livers of a group of dogs subjected to identical experimental procedures²⁰.

In view of this last observation we are led to another conclusion—that the end stages of hepatic cirrhosis in dogs treated in different ways may be pathologically similar, even though the causation of the lesions and their genesis may be quite different. It seems that no conclusions concerning either the cause or the genesis of a cirrhotic process can be drawn from an examination of a single section of a liver taken at a single moment during the end stages of the disease.

13 Deysach, L. J. *Marquette M. Rev.* **7** 139, 1943.

14 Himsworth, H. P., and Glynn, L. E. *Clin. Sc.* **6** 235, 1948.

15 (a) Gillman, J., Gillman, T., Mandelstam, J., and Gilbert, C. *Brit. J. Exper. Path.* **26** 67, 1945. (b) Gillman, J., and Gillman, T. *Malnutrition in South African Negroes*, New York, Grune & Stratton, Inc., to be published.

16 Lucke, B. *Am. J. Path.* **20** 595, 1944.

17 Gillman, J., Gilbert, C., and Gillman, T. *South African J. M. Sc.* **12** 99, 1947.

18 Lindsay, S., Entenman, C., and Chaikoff, I. L. *Arch. Path.* **45** 635, 1948.

19 Kristal, J. *South African J. M. Sc.* **12** 47, 1947. Gillman and Gillman^{15b}.

20 Chaikoff and others, footnotes 3 and 4 h.

SUMMARY

Three pathogenetically distinct forms of connective tissue hyperplasia or hepatic fibrosis have been induced experimentally in dogs (1) centrolobular or peri-hepatic vein fibrosis, (2) periportal fibrosis, (3) diffuse interstitial or intralobular fibrosis

The final pathologic pictures observed following the development of the three forms of fibrosis may be similar. The end picture of each is determined not only by the site of initiation of the fibrosis but also by the extent to which the liver is affected, the speed of development of the initial lesion and the degree to which the first type of fibrosis is complicated with focal necrosis, lobar or lobular hypertrophy or atrophy, and the supervention of additional forms of fibrosis.

While fibrosis, especially the centrolobular type, was frequently associated with severe fatty change of the hepatic epithelium or occasionally with central hemorrhagic necrosis, nevertheless it was also seen without being obviously related to hepatocellular damage.

Factors other than extensive degeneration or destruction of hepatic epithelium may play a critical role in determining the site of onset of hepatic fibrosis. Attention is drawn to the possible role of circulatory changes in the genesis of hepatic fibrosis. The relative independence in reactivity of the parenchymal cells and the supporting tissue of the liver is indicated.

Different forms of fibrosis can occur in animals treated in an identical fashion, and even in different parts of a single liver. No correlation could be established between the type of fibrosis or the terminal cirrhosis and the experimental procedure used to induce the hepatic changes.

ADENOMA OF CERUMINOUS GLAND IN THE DOG

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A CASE of adenoma of the ceruminous glands has a twofold interest. Only a few neoplasms of the ceruminous glands of man or animals have been reported and it is rare to find a tumor of cutaneous glands of either apocrine or eccrine type in which the growth can be traced to the secretory elements in distinction from the ducts. Three recent reviews of the subject are those of Warren and Gates,¹ Gates, Warren and Warvi,² and Adler and Sommer.³ Willis⁴ found 1 dog with ceruminous adenoma among 204 dogs with various tumors. The tumor which is now reported has been reviewed by David Coffin,⁵ of the Animal Angell Memorial Hospital of Boston, R M Mulligan,⁵ of Denver, William Feldman,⁵ of Rochester, Minn, Raymond O Dart⁵ and T C Jones,⁵ of Washington, D C. They have all agreed that it is an adenoma of ceruminous gland origin.⁶

In a seeing eye dog being treated for stubborn ulcerative otitis externa, two apposed growths developed in the cartilaginous portion of the external acoustic meatus approximately 2.5 cm from the external orifice on the medial and lateral aspects of the canal. They were completely excised.

Two roughly spherical pieces of tissue partly covered by patches of thickened gray skin were received in 4 per cent formaldehyde solution. Each measured 1.2 by 1 by 0.7 cm. The cut surface showed grayish brown, firm, lobulated, glistening tissue dotted with yellowish flecks and reddish brown foci of hemorrhage.

Microscopically the neoplasm, which lay in the upper part of the corium, compressing but not joining the epidermis, appeared to arise from the secretory alveoli, although there were a few foci which suggested that the ducts also might be involved in the new growth. The tumor was composed of acinous and solid epithelial elements, with a predominance of the acinous component. These acini varied greatly in size. A few approached the dimensions of the normal alveoli,

From the Harvard Cancer Commission Laboratory of Pathology and the State Tumor Diagnosis Service

1 Warren, S, and Gates, O. *Am J Path* **17** 821, 1941

2 Gates, O, Warren, S, and Warvi, W. *Am J Path* **19** 591, 1943

3 Adler, H S, and Sommer, I. *Arch Otolaryng* **39** 533, 1944

4 Willis, R A. *Pathology of Tumours*, St Louis, C V Mosby Company, 1948, p 95

5 Personal communication to the author.

6 The specimen and the clinical history were provided by Paul R Granholm, D V M, 348 Boston Post Road, Weston, Mass.

but most of them were larger and a few, five or six times the normal size, could be designated as cysts (fig 1) The solid growth (figs 2 and 4) was by comparison insignificant and appeared to result mainly from proliferation of cells within the alveoli rather than from infiltration of cells into surrounding stroma

The cells of the tumor in the main resembled the normal secretory epithelium of the ceruminous glands (fig 5) The commonest form was a cuboidal cell, either low or tall The free surface was smooth and rounded, with the lipping characteristic of apocrine structures and occasionally a frayed border indicating active secretion The nucleus tended to lie in the basal part of the cytoplasm It was pale, and the rather large nucleolus was therefore prominent The cytoplasm was slightly refractile and eosinophilic, sometimes pale but more often staining intensely Products of secretion, such as fat, pigment globules and deeply acidophilic granules, were frequently a prominent feature of the cytoplasm (figs 3 and 4) Amorphous golden brown material, eosinophilic granules and a rather amorphous hyaline substance lay free in the lumens of alveoli and in the stroma, where they attracted histiocytes Special stains showed that the golden brown pigment granules contained iron, while the less refractile paler pigment, apparently intimately bound to the lipid, did not

Besides the obvious secretion of this tumor, the myoepithelial cells in a few of the well differentiated alveoli also indicated that it was derived from the secretory portions of the ceruminous gland These myoepithelial cells were quite insignificant as compared with their appearance in the normal gland and were not recognized in the poorly differentiated alveoli The more solid growth appeared to be the result of proliferation of cells lining alveoli rather than of invasion of the surrounding stroma Most striking was a papilliform growth within dilated acini, the cells being tall columnar, with colorless or faintly basophilic cytoplasm Squamous metaplasia of alveolar cells occurred frequently and sometimes progressed to complete keratinization, but epithelial pearls and intercellular bridges were not seen

There was a moderately dense fibrous stroma showing areas of myxomatous change which produced the effect of an infiltrating growth In the absence of a capsule a growth so variable in structure is apt to appear more cancerous than it is The absence of invasion of blood vessels and lymphatic channels, the few mitotic figures and the active secretion suggest a benign tumor

We have had an opportunity to examine four other tumors of the same type These are enumerated

Slides of 3 tumors of ceruminous glands from the files of the Registry of Veterinary Pathology of the Army Institute of Pathology were supplied by Brig Gen Raymond O Dart, Medical Corps, and Major T C Jones, Veterinary Corps, United States Army

Accession 183438, a tumor from the external auditory canal of a cat was submitted by Dr R F Vigue, of Sanford, Maine

Accession 185379, a tumor from the external auditory canal of a male Doberman pinscher was submitted by Dr George M Stewart, of Brentwood, Md, this tumor was removed in January 1947, it recurred a month later and, following this excision, has recurred a second time

Accession 185427 from a bull terrier 8 years old was submitted by Dr S W Stiles, of Falmouth Foreside, Maine, there was no recurrence a year later

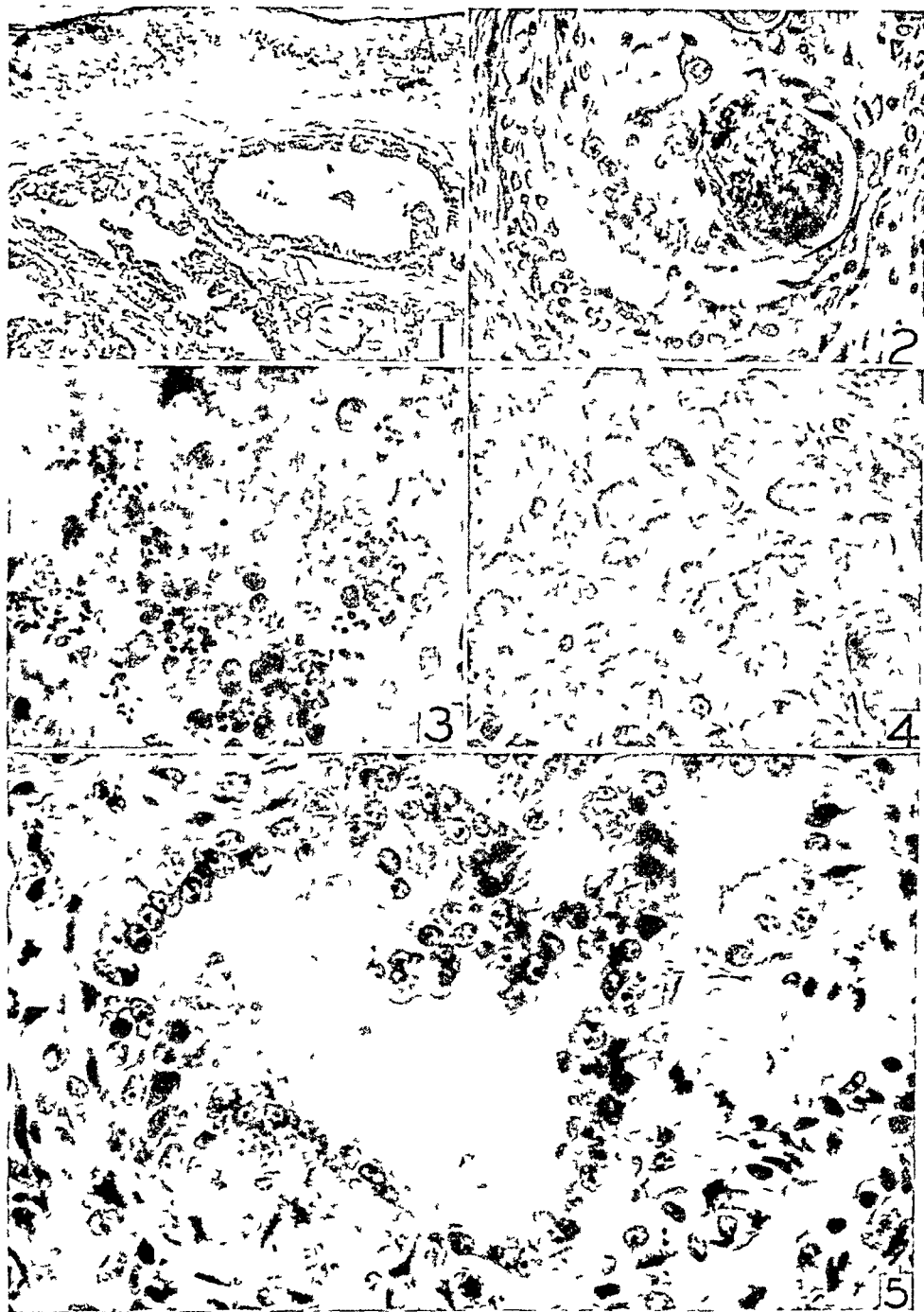


Fig 1—Cystically dilated glands with papilliform projections $\times 50$

Fig 2—Squamous metaplasia in a duct $\times 200$

Fig 3—Stromal histiocytes containing fat Sudan IV, $\times 500$

Fig 4—Stromal histiocytes $\times 500$

Fig 5—Gland showing "lipping" of the cells, denoting active secretion. Droplets of secretion lie free in the lumen $\times 500$

A paraffin block from a fourth tumor⁷ was supplied by Dr R M Mulligan, professor of pathology at the University of Colorado School of Medicine. This tumor from a 15 year old male fox terrier was submitted by Dr L R Phillips and Dr B S Burkhardt, both of Lakewood, Colo.

Sections from these four tumors show essentially the same characteristics as the tumor presented in this report except that little solid growth occurred and there was no squamous metaplasia.

COMMENT

This canine ceruminous gland adenoma, though not encapsulated, appears to have represented an expansile rather than an actively infiltrating growth. The greater part was well differentiated and exhibited the functional characteristics of normal secreting acini of ceruminous glands.

It is of particular interest that some elements, namely, the papillary, almost mucinous type of columnar epithelium and the squamous keratinized masses of cells, which commonly occur in syringocystadenoma as a result of proliferation of epithelium of ducts, were in this case clearly derived from the secreting alveolar epithelium. It is rare indeed to find convincing evidence to implicate the secretory cells of cutaneous glands in a neoplastic process. This tumor therefore was unusual because it was a functioning tumor of ceruminous glands and because it demonstrated the fact that two different types of epithelium, secreting epithelium and lining epithelium of ducts, may through metaplasia produce the same form of tumor.

SUMMARY

A tumor of the ceruminous glands of a dog is here presented in detail. Four similar tumors of 3 dogs and a cat have been reviewed and are reported for the first time. Tumors of ceruminous glands have received little study. They are exceedingly rare in man, but several veterinarians have expressed the opinion that they are of common occurrence in the dog.

⁷ Dr Mulligan's case bears the number 47R-304 under Research Grant C-380-R of the National Cancer Institute of the United States Public Health Service. Dr Mulligan's recent review of 120 canine neoplasms (*Arch Path* 45:216, 1948) does not include ceruminous gland tumors.

EFFECT OF EXPERIMENTAL THIAMINE DEFICIENCY ON THE HEART OF THE RHESUS MONKEY

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AND

L D GREENBERG, Ph D

SAN FRANCISCO

DURING the past several years progress in nutritional research has been rapid. With clarification of the chemical nature of essential food factors, particularly those of the vitamin B complex, it has become possible to study the influence of single deficiencies in the monkey with a degree of precision heretofore unattainable. It seems most timely to restudy the vitamin deficiencies in a primate in which the metabolic processes would be expected to approximate closely those of man. We have therefore undertaken systematic studies of the vitamin deficiencies in the rhesus monkey. The major lesions developing in the central nervous system and the heart as a result of thiamine depletion have been reported in abstract,¹ and the precise character and distribution of the degenerative lesions developing in the nuclear structures of the central nervous system have been detailed separately.² The present report is concerned primarily with the histopathologic changes seen in the heart muscle in thiamine deficiency.

METHOD

The diet used was a modification of the M-3 diet of Waisman and associates.³ The composition of the diet, together with notes on the clinical behavior of the animals, biochemical observations on thiamine metabolism and influences on hemopoiesis have been reported.⁴

Particular mention should be made of the fact that of the 7 animals studied, 3 were subjected to two episodes of acute depletion and 2 others to three such episodes, the total periods ranging from one hundred to one hundred and seventy

From the Division of Pathology, University of California Medical School

This investigation was supported by a donation from the California Fruit Growers Exchange and the A. B. Miller Fund

1 Rinehart, J. F., Greenberg, L. D., and Friedman, M. Abstracted, *Am. J. Path.* **23**: 879, 1947. Rinehart, J. F., Friedman, M., and Greenberg, L. D. *Tr. Am. Neurol. A.* **72**: 174, 1946.

2 Rinehart, J. F., Friedman, M., and Greenberg, L. D. *Arch. Path.*, to be published.

3 Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F. *J. Nutrition* **26**: 205, 1943.

4 Rinehart, J. F., Greenberg, L. D., and Ginzton, L. L. *Blood* **3**: 1453, 1948.

days One animal was maintained in a deficient state by administration of repeated suboptimal doses of thiamine for a total experimental period of sixty days The other animal was examined at the end of a single depletion period of fifty days There is no doubt that the procedure of producing recurrent depletion has augmented the pathologic changes

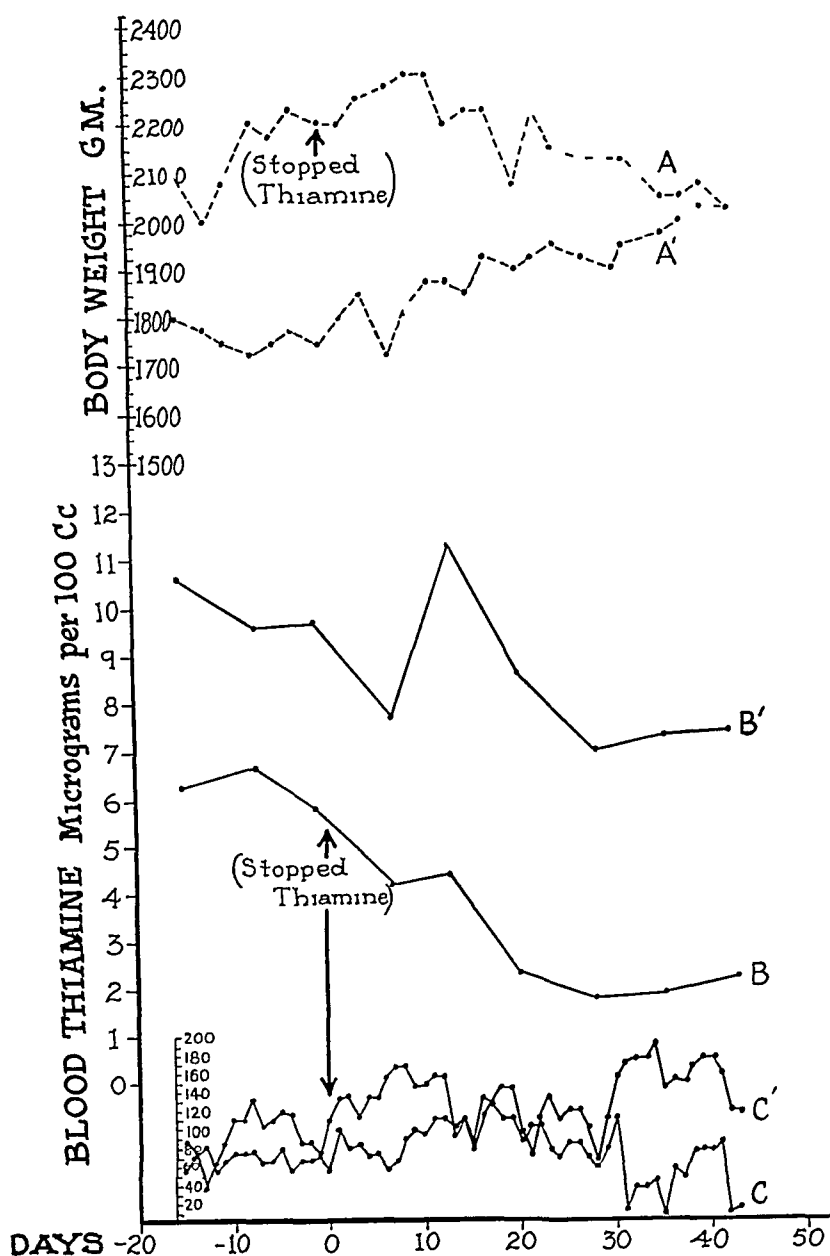


Fig 1—*A* and *A'* weight curves of a thiamine-deficient rhesus monkey and its control, respectively *B* and *B'* blood thiamine levels of deficient monkey and control *C* and *C'*, food consumption curves of deficient monkey and control

When thiamine is removed completely from the diet, the animals will show diminished consumption of food after two weeks with concurrent loss of weight

This observation has been made repeatedly. Curves showing weight, food consumption and blood thiamine concentration for deficient and control animals are presented in figure 1. It is evident under the controlled conditions of observation that the animal is in fact beginning to suffer from thiamine deficiency after two to three weeks of depletion. While pathologic observations have not been made at this stage, we are confident that present methods of histologic examination would not reveal lesions. When the deficient state is prolonged, the animals become apathetic, inactive and progressively weaker. This is followed by ataxia and at times ptosis and tremors. Several animals have shown dyspnea on exertion when removed from the cage for examination. Even in such advanced states of depletion administration of thiamine will produce dramatic improvement in strength, locomotion, appetite and reactivity. Control animals maintained on the same basic diet with supplements of thiamine have remained in vigorous good health.

We have recently reported a sensitive method for the determination of the thiamine levels of blood and tissues⁵. The blood thiamine of the monkey fed an adequately supplemented diet ranges from 5.5 to 10 micrograms per hundred cubic centimeters, while in the depleted animal it is from 2 to 4 micrograms (fig. 1).

PATHOLOGIC CHANGES IN THE HEART

Gross examination of the hearts showed dilatation of the right auricle and ventricle in 5 of the 7 animals and at times some dilatation of the left ventricle (fig. 1).

Microscopic examination revealed that significant cardiac lesions had developed in 4 of the 7 animals. The lesions were of two types. One consisted of focal necrosis of the heart muscle as shown in figures 2 *A* and *B*. This lesion was of variable extent. It appeared to represent slow degeneration or necrobiosis of muscle fibers rather than acute necrosis. There were reactive hyperplasia of the reticular stromal cells and some leukocytic infiltration. In the animal showing the most extensive necrosis of this type, the lesion was chiefly beneath the endocardium and of such extent that had it healed it probably would have occasioned some subendocardial fibrosis. The other type of cardiac lesion was seen in the same 4 animals. Although somewhat elusive, it was perhaps more distinctive of thiamine deficiency. It consisted of swelling and development of large clear areas in the cytoplasm of certain subendocardial muscle fibers, accompanied by hypertrophy and hyperchromatism of associated nuclei. At times there appeared also to be a degree of interstitial edema. The character of the lesion is well shown in figures 2 *D* and *E*. The lesion was most frequently seen in the left ventricle but was observed also in the right ventricle. The size and the distribution of abnormal fibers leave little doubt that they were part of the specialized myocardial fibers of the conduction system. We have not encountered lesions of either type in the auricles.

⁵ Greenberg, L. D., and Rinehart, J. F. Proc. Soc. Exper. Biol. & Med. 59: 9, 1945.

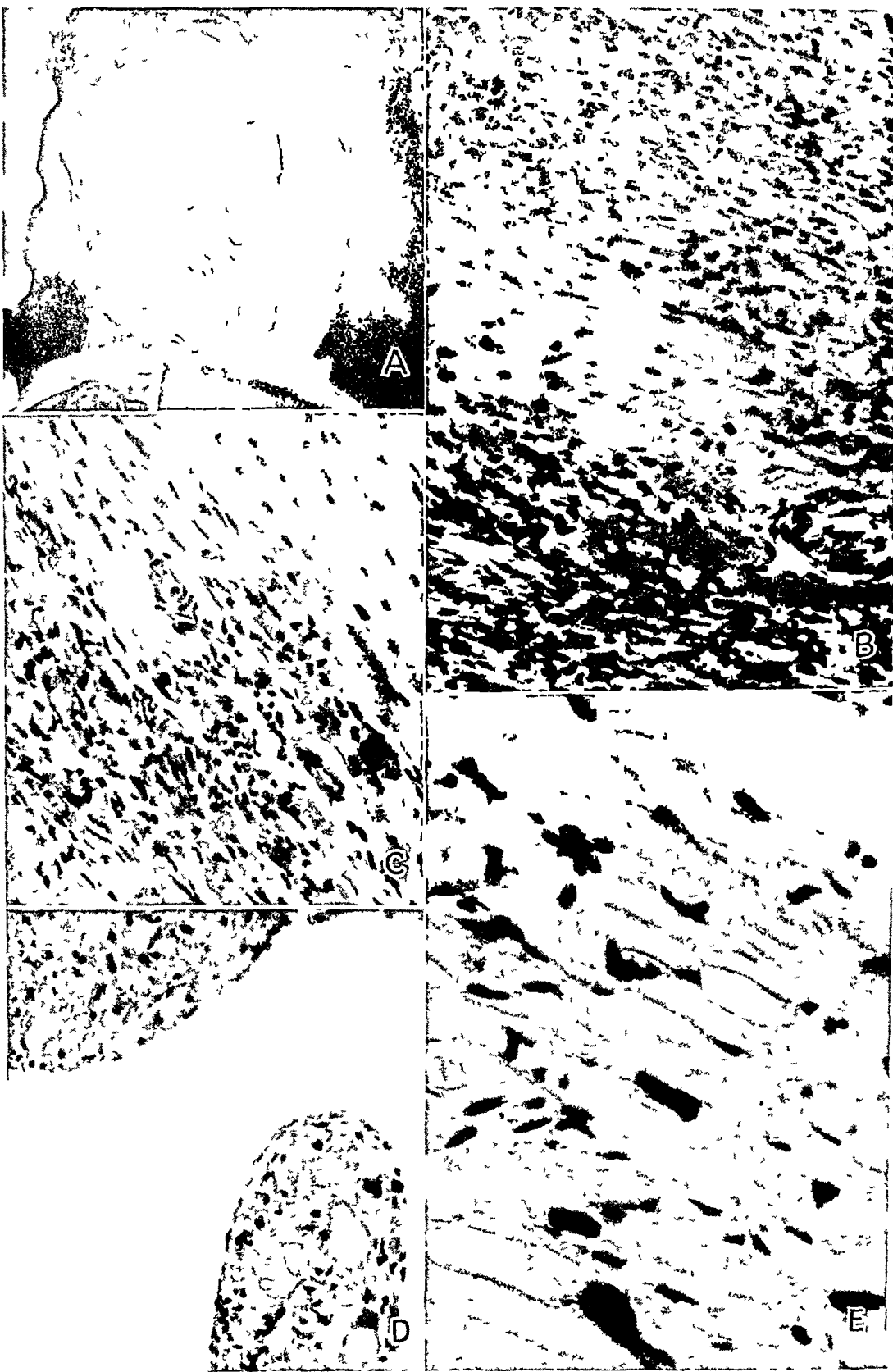


Fig 2—*A*, gross appearance of the heart in acute recurrent thiamine deficiency. Note dilatation of both ventricles, particularly of the right. *B*, extensive subendocardial degeneration and necrosis of heart muscle, $\times 205$. *C*, small focus of myocardial necrosis, $\times 205$. *D*, striking “hydropic” swelling of subendocardial muscle fibers of the conduction system, $\times 205$. *E*, myocardial fibers beneath the endocardium, $\times 410$. Note irregular hyperchromatic nuclei and pallor and swelling of cytoplasm.

COMMENT

That a functional cardiovascular defect occurs in thiamine deficiency in man and animals is well established. The exact nature of this defect, however, has not been clarified, and the morphologic manifestations have remained somewhat obscure. Follis and associates⁶ recently reviewed pertinent literature and reported focal necroses of the myocardium of swine subjected to thiamine deficiency. Van Etten, Ellis and Madsen⁷ also described such lesions of pigs given a diet supplemented by sulfite-treated liver and whey. Lesions of similar character have been observed in pigeons by Swank⁸ and in 3 of 14 dogs subjected to thiamine deficiency by Swank, Porter and Yoemans.⁹ Porto and de Soldati¹⁰ and de Soldati¹¹ reported myocardial "infarcts" of a dog subjected to thiamine deficiency and hydropic degeneration of myocardial and conduction fibers of other animals. In de Soldati's monograph focal necroses of the heart muscle of the thiamine-deficient dog are described and illustrated, as well as perinuclear vacuolar degeneration of muscle fibers, including those of the conduction system. Focal myocardial necroses associated with hemorrhage were noted by Evans, Carlson and Green¹² in foxes dying of Chastek paralysis, which is almost certainly dominantly a thiamine deficiency syndrome. They also noted enlargement of nucleoli and intracellular edema of cardiac muscle cells in some instances. More recently Ashburn and Lowry¹³ observed myocardial necroses in rats subjected to prolonged thiamine deficiency. These lesions were dominantly in the auricles. Forty-seven of 60 rats had "necrosis of muscle fibers, cellular infiltration and proliferation or evidence of previous damage, such as decreased number or absence of muscle fibers and slight to moderate fibrosis." Similar lesions were seen in the ventricular myocardium in only 7 rats, and in these the involvement was minimal.

It appears that focal necroses of the myocardium have been observed in many species of animals subjected to thiamine deficiency. The vacuolar or hydropic degeneration of the fibers of the conduction system appears to be more distinctive of thiamine deficiency. Lesions

6 Follis, R. H., Jr., Miller, M. H., Wintrobe, M. M., and Stein, H. J. *Am J Path* **19** 341, 1943.

7 Van Etten, C., Ellis, N. R., and Madsen, L. L. *J Nutrition* **20** 607, 1940.

8 Swank, R. L. *J Exper Med* **71** 683, 1940.

9 Swank, R. L., Porter, R. R., and Yoemans, A. *Am Heart J* **22** 154, 1941.

10 Porto, J., and de Soldati, L. *Rev Soc argent de biol* **15** 426, 1939.

11 de Soldati, L. *Los trastornos circulatorios de la avitaminosis B₁*, Buenos Aires, El Ateneo, 1940.

12 Evans, C. A., Carlson, W. E., and Green, R. G. *Am J Path* **18** 79, 1942.

13 Ashburn, L. L., and Lowry, J. V. *Arch Path* **37** 27, 1944.

of this type in man have been recorded. Thus Wenckebach¹⁴ described and illustrated an apparently identical pathologic change in his classic study of the beriberi heart. Weiss and Wilkins¹⁵ were responsible for directing attention to the not infrequent occurrence of beriberi heart disease in the United States. They made pathologic examinations in a few cases and recorded "hydropic" degeneration of muscle fibers and illustrated changes of subendocardial conduction fibers that are analogous to those detailed by Wenckebach and similar to those described in this report. Weiss and Wilkins questioned the specificity of the "hydropic degeneration" because such lesions were said to be seen in other patients not known to have thiamine deficiency.

SPECIFICITY AND SIGNIFICANCE OF THE LESIONS

The focal myocardial necroses, although frequently encountered in experimental thiamine deficiency, can scarcely be considered specific. As pointed out by Follis, analogous lesions have been seen in potassium deficiency and in scurvy. It is also noteworthy that such focal necroses have not been mentioned in descriptions of the human beriberi heart. The peculiar "hydropic degeneration" of the conduction fibers is, we believe, in its fully developed stages quite distinctive. It has been demonstrated in 4 of the animals of this study and corresponds to the lesion that has been observed in human deficiency. It should be borne in mind that the conduction fibers are somewhat larger than other heart muscle fibers and normally show larger nuclei and paler cytoplasm. In a certain sense the pathologic change is an exaggeration of a normal cytologic feature of the conduction fiber. The lesion is consequently somewhat elusive and must be well developed to be characteristic. A somewhat similar appearance was noted in 1 animal subjected to folic acid deficiency but not in controls or other animals undergoing equally prolonged periods of inanition incident to other deficiencies.

While both lesions are definite, it is problematic whether they are fully responsible for the circulatory defect in thiamine deficiency. Both Wenckebach and Weiss and Wilkins have presented evidence that there is a peripheral arteriolar dilatation in beriberi which is so marked that it acts similarly to an arteriovenous shunt. The latter have shown that the circulation time is shortened in beriberi and that there is decreased peripheral utilization of oxygen. Weiss suggested that this phase of the circulatory defect is probably related to disturbances of nerve functions, possibly in the central nervous system. Our observations of widespread and significant lesions in many of the nuclear structures of the central

14 Wenckebach, K. F. *Das Beriberi-Herz, Morphologie, Klinik, Pathogenese*, Berlin, Julius Springer, 1934.

15 Weiss, S., and Wilkins, R. W. *Ann Int Med* **11** 104, 1937.

nervous system in thiamine-deficient monkeys² lend strong support to this concept. Unfortunately, we did not study the circulation time or the peripheral utilization of oxygen in our animals.

The only other recorded experiments in which thiamine deficiency was produced in the monkey and which may be considered to be uncomplicated with other deficiencies are those recently reported by Waisman and McCall¹⁶. They noted decreased consumption of food, loss of weight, muscular weakness, loss of reflexes, convulsions, incoordination, increasing cachexia and signs of cardiac insufficiency as the clinical manifestations of thiamine deficiency in the rhesus monkey. The blood "pyruvic acid" level was sharply elevated in the thiamine-deficient animal. Electrocardiographic tracings showed lowered heart rate, decreased amplitude of the R wave and inversion of the T waves. The few electrocardiographic tracings which we have made are similar. Histopathologic studies were not reported.

It would seem desirable to determine the concentration of thiamine in the blood and possibly skeletal muscle of patients whose symptoms suggest thiamine deficiency and to correlate studies of the blood and tissue contents of thiamine with histopathologic changes in cases of otherwise unexplained circulatory failure such as might be encountered in the coroner's jurisdiction. Likewise, careful physiologic studies of the thiamine-deficient monkey should serve to clarify the nature of the significant circulatory defect.

SUMMARY

Rhesus monkeys subjected to recurrent episodes of thiamine deficiency show dilatation of the right side of the heart and focal necroses in the heart muscle which are analogous to lesions described in many other species. A second and probably more distinctive lesion is a peculiar degenerative change occurring in the fibers of the conduction system, manifested by irregular hypertrophy of nuclei and "hydropic degeneration" of the cytoplasm. It is problematic whether these lesions are fully responsible for the circulatory defect of thiamine deficiency. The clinical observations of marked arteriolar dilatation in human thiamine deficiency suggest the important influence of a vasomotor factor. The finding of widespread degenerative changes in the nuclear structures of the brain stem in the animals of this experiment² lends support to this concept.

16 Waisman, H. A., and McCall, K. B. *Arch Biochem* 4: 265, 1944.

Case Reports

ONCOCYTOMA OF THE PAROTID GLAND

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HAMPERL¹ originated the term "onkocyte," although he gave credit to Shaffer for having first described these cells in the salivary glands, the pharynx, the trachea and the esophagus as "granular swollen cells." The name "onkocyte" was suggested since the cell is characterized by the constitution of its cytoplasm and consequent enlargement. Further investigation concerning this type of cell revealed that certain tumors of the salivary glands were made up largely of oncocytes.² Ackerman³ gathered 7 well documented cases of oncocytoma of the salivary glands from the literature and added 1 of his own. The case reported here is one of a "mixed" type of tumor in which the epithelial component is made up of oncocytes.

REPORT OF CASE

A Negro man aged 67 was seen in the tumor clinic in January 1948. Beneath the right ear there was a painless firm lump which had gradually increased in size since the patient first noticed it two years previously. It was approximately 5 cm in diameter and was not attached to skin or bone, and was movable.

The tumor was widely excised, the specimen including a generous portion of apparently normal parotid gland. It was 7 by 5 by 3.5 cm and weighed 35 Gm. The tumor was irregularly rounded and rather firm and appeared fairly well encapsulated. On sectioning, the surface was gray-brown and homogeneous except for two small areas which were soft and cystic. Numerous blocks were selected for microscopic examination and stained with hematoxylin and eosin.

The tumor had a thick, though incomplete, connective tissue capsule. For the most part, the cells were uniform and arranged in columns and pseudoacinous fashion without formation of true glands or ducts. The groups of cells were separated by delicate strands of connective tissue. The individual cells were large, with abundant bright acidophilic granular cytoplasm. The nuclei were round, with evenly distributed chromatin, nucleoli were quite prominent. The cells had a striking resemblance to hepatic or adrenal cells.³ No mitotic figures were found. In a few areas there were groups of smaller cells that tended to form ducts, these areas contained more stroma, which varied from hyaline through mucoid to cartilaginous. The portion of the gland adjacent to the tumor was normal.

From the Department of Pathology, University of Louisville School of Medicine

1 Hamperl, H. Virchows Arch f path Anat **282** 724, 1931

2 Hamperl, H. Ztschr f mikr-anat Forsch **27** 26, 1931

3 (a) Ackerman, L. V. Arch Path **36** 508, 1943 (b) McFarland, J. Am J M Sc **174** 362, 1927

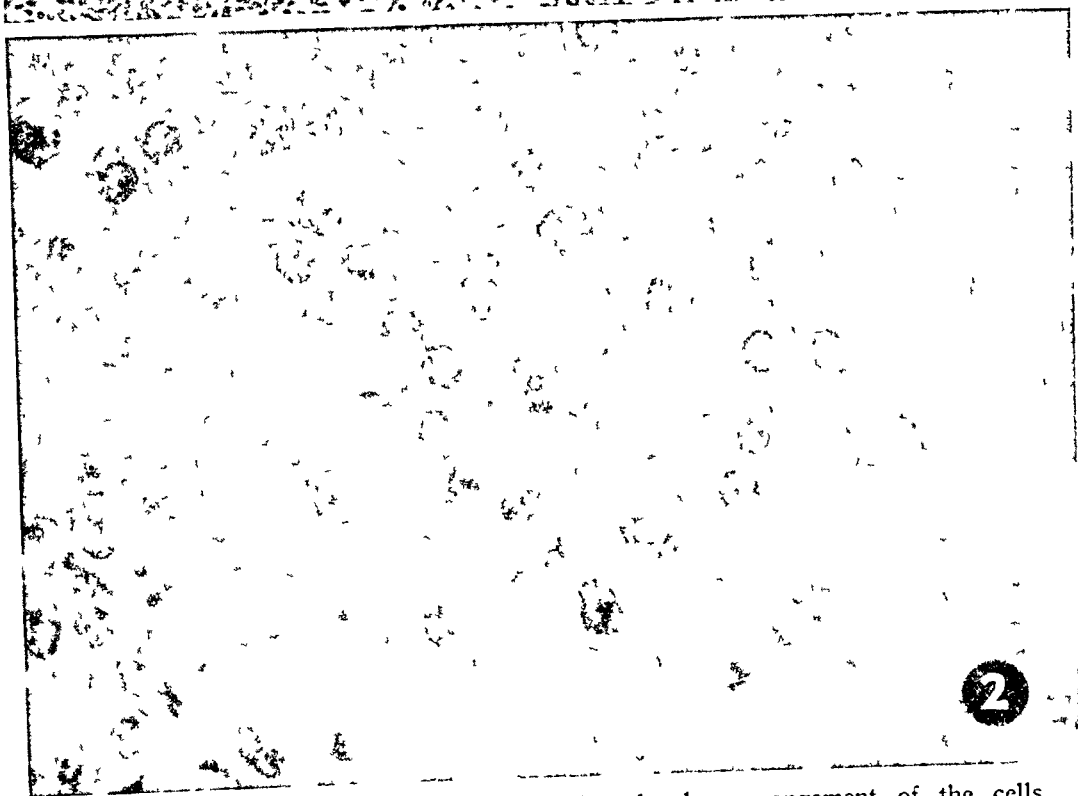


Fig 1—Low magnification showing the alveolar arrangement of the cells and the paucity of stroma

Fig 2—High magnification showing the large cells with superficial resemblance to adrenal or liver cells

COMMENT

Oncocytes are epithelial cells characterized by a peculiar honey-combed acidophilic granular cytoplasm. They resemble the cells of the parent organ but are much larger, those found in the parotid gland having rather a close resemblance to hepatic or adrenal cells. Their exact origin is not known, they seem to represent a new type or form of an already differentiated cell,⁴ or what amounts to redifferentiation. These cells appear with sexual maturity and increase with advancing age, which suggests that the peculiar appearance is due to senility of the cell. Hamperl² found signs of cell proliferation but always with amitotic cell division, whereas in physiologic regeneration of salivary glands mitoses are observed. In addition to the salivary glands, oncocytes have been demonstrated in the pancreas, the parathyroid glands, the hypophysis, the thyroid gland, the fallopian tubes, the liver, the testes⁴ and the bronchi.⁵ Stout⁵ established that oncocytes are present in the bronchi of adults and not present in fetal bronchi. He considered that these cells may possibly be the stem cells of bronchial adenoma.

Without entering into the controversy as to the origin of the so-called "mixed" tumors I believe it is possibly of some significance that in the tumor reported here the small areas of cartilaginous stroma occurred only in a few regions, where the cells were smaller and tended to form ducts, and was conspicuously absent from the bulk of the tumor, in which the epithelial component was purely oncocytic.

The interval since excision of this tumor is too short for one to predict the ultimate outcome, but at the time of writing there is no recurrence and no histologic evidence of cancer. This is in accord with the previously reported cases, in all of which the tumor behaved as a benign neoplasm.⁶

SUMMARY

Oncocytoma of the parotid gland is a rare tumor with a striking, easily recognized histologic structure. In the case reported here the tumor contained small cartilaginous areas similar to those found in the so-called "mixed" tumors.

4 Hamperl, H. Virchows Arch f path Anat **298** 327, 1936

5 Stout, A. P. Arch Path **35** 803, 1943

6 Hamperl, H. Virchows Arch f path Anat **300** 46, 1937 Ackerman^{3a}

Notes and News

Appointments, etc—George J Rukstinat has been appointed professor of clinical pathology in Loyola University, Chicago

P R Beamer, Washington University Medical School, St Louis, has been appointed professor of microbiology and immunology and associate professor of pathology at the Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N C

Hugh W Jones, formerly chief of pathology, Walter Reed General Hospital, Washington, D C, has become director of the department of pathology and clinical laboratories of the Mason Clinic in Seattle, Wash

Death—Felix d'Herelle, well known for his work on bacteriophage and his writings in English and French on immunity in infectious diseases, died in Paris, Feb 22, 1949 He was born in 1873, in Montreal, where he received his M D degree He was chef de laboratoire in the Pasteur Institute in Paris from 1914 to 1921 During this time and later he frequently served on special missions to various countries and was professor of protobiology in Yale University from 1928 to 1933, when he founded his own laboratory in Paris

Training for Cancer Research—The University of California Medical School announces a postgraduate course in the medical aspects of nuclear energy, August 29 to September 3, at the Medical Center, San Francisco Joseph G Hamilton, director of the Crocker Laboratory, University of California, will be chairman of the course A detailed program will be mailed on request addressed to Stacy R Mettier, Head of Postgraduate Instruction, Medical Extension, University of California Medical Center, San Francisco 22

The Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, has established a residency for a minimum of six months for training of pediatricians in neoplastic diseases of childhood Address Director of Pediatric Service, Memorial Hospital, 444 East Sixty-Eighth Street, New York 21

The American Cancer Society announces the availability of the Damon Runyon Clinical Research Fellowships which it administers on recommendation of the National Research Council Open to men and women with the M D degree, the fellowships will, in most cases, provide a period of training in a hospital under the guidance of a qualified clinical investigator but may also be awarded for training in a basic science, provided that such training is directed toward preparing the fellow for clinical cancer research The annual stipend may vary from \$2,500 to \$6,000, in most instances it will not exceed \$4,000 Applications may be submitted at any time and should be addressed to the Executive Secretary, Committee on Growth, National Research Council, 2101 Constitution Avenue, Washington 25, D C

Training for Teaching and Research—The American College of Physicians announces that a limited number of fellowships in medicine will be available from July 1, 1950 to June 30, 1951 These fellowships are designed to provide an opportunity for training in research either in the basic medical sciences or in the application of these sciences to clinical investigation They are for the benefit of physicians who are in the early stages of preparing for a teaching and investigative

career in internal medicine The stipend will be from \$2,200 to \$3,000 Application forms will be supplied on request to the American College of Physicians, 4200 Pine Street, Philadelphia 4, and must be submitted in duplicate not later than Oct 1, 1949

Society News—Newly elected officers of the Alabama Association of Pathologists for the year 1949-1950 are J A Cunningham, president, J S P Beck, vice president, R D Baker, member of executive committee and A E Casey, secretary-treasurer, Birmingham

The American Association of Pathologists and Bacteriologists at its recent meeting in Boston elected Drs Shields Warren, Alan R Moritz and Sidney Farber, all of Boston, president, secretary and treasurer, respectively

Revised Edition of Motion Picture Reviews Now Available—The Committee on Medical Motion Pictures has completed the first revised edition of the booklet entitled "Reviews of Medical Motion Pictures" It now contains all the film reviews published in *The Journal of the American Medical Association* to January 1, 1949 It also includes a classified table of contents, as well as a list of motion pictures available through the Motion Picture Library, American Medical Association

The purpose of the reviews is to provide a brief description and evaluation of motion pictures which are available to the medical profession Each film is reviewed and commented on by competent authorities

Copies are available on request from

Committee on Medical Motion Pictures
American Medical Association
535 North Dearborn Street
Chicago 10, Illinois

Books Received

PSYCHODYNAMICS AND THE ALLERGIC PATIENT By Harold A. Abramson, M.D., associate physician for allergy, Mount Sinai Hospital, New York, consulting physician for allergy, Sea View Hospital, Staten Island, N. Y., assistant professor of physiology, Columbia University, New York. Panel Discussion: Rudolph L. Baer, M.D., Ethan Allan Brown, M.D., Hal M. Davison, M.D., O. Spurgeon English, M.D., Frank Fremont-Smith, M.D., J. A. P. Millet, M.D., M. Murray Peshkin, M.D., Homer E. Prince, M.D., Sándor Rado, M.D., Edward Weiss, M.D. An official publication of the American College of Allergists. Pp. 81, with 6 illustrations. Price \$2.50. St. Paul and Minneapolis, Minn.: Bruce Publishing Company, 1948.

This book is a significant contribution to the growing literature dealing with the psychosomatic aspect of allergic disorders. It is particularly important because it is directed to the attention of the practicing allergist and succeeds in demonstrating in a conclusive manner the significance of emotional factors in the total etiologic picture of the allergic patient.

The book is a report of a panel discussion on the subject "Psychodynamics and the Allergic Patient" held under the auspices of the American College of Allergists at its annual meeting in Atlantic City, N. J., June 8, 1947. It contains Abramson's paper on the subject as well as the discussion by members of the panel. Although the paper and the discussion do not take into consideration the more specific emotional dynamics relating to the various allergic manifestations that have been worked out and reported by psychoanalysts, the book does call the allergist's attention to the need for a revision of the attitude toward both the theory and the practice of the treatment of the allergic patient, indicating that immunologic methods are not enough and that advantage must be taken of the advances made in psychoanalytic psychology.

Abramson's paper is also interesting from a historical point of view. He shows that many observers going back hundreds of years were aware of the emotional factors in hay fever and asthma. The members of the panel contributed excellent discussions on the subject and these are properly recorded in the book. In addition to emphasizing the psychosomatic approach, the problem of who is to treat the allergic patient is touched on. Another topic of related interest discussed was the manner of how much and what kind of training in the psychosomatic approach should be given to the allergist. The book is a fine beginning and fills a need in the library of the physician and particularly the allergist.

FETAL AND NEONATAL DEATH. A SURVEY OF THE INCIDENCE, ETIOLOGY AND ANATOMIC MANIFESTATIONS OF THE CONDITIONS PRODUCING DEATH OF THE FETUS IN UTERO AND THE INFANT IN THE EARLY DAYS OF LIFE. By Edith L. Potter, M.D., Ph.D., associate professor in the department of obstetrics and gynecology, University of Chicago, and pathologist at the Chicago Lying-In Hospital, and Fred L. Adair, M.D., Mary Campau Ryerson professor emeritus, University of Chicago. Pp. 173, with 38 illustrations and 19 tables. Price \$3.75. Chicago: The University of Chicago Press, 1949.

The first edition of this book appeared in 1940. In this, the second, edition the authors have added pertinent subject matter on the Rh factor and its influence on the fetus and the newborn child, and on the effects of maternal rubella (German measles) on the fetus.

In the preface Dr Potter emphasizes that in the nine years which have elapsed since the book was first written, there has been "a widespread increase in the amount of attention centered on pregnant women and on the fate of their unborn and newly born offspring" "In spite of the fact that there were almost six hundred thousand more births in 1945 than in 1937", there were about a thousand fewer stillbirths and fourteen thousand fewer deaths under one month of age. The combined rate per 1000 live births had dropped from about 66 in 1937 to 48 in 1945."

The book is divided into five chapters. Chapter I deals with the normal fetus and infant. This chapter includes authoritative information on fertilization and early development, measurements and weights. This is followed by a detailed discussion of the developmental anatomy of each organic structure of the body during fetal life and in the newborn infant. Chapter II discusses the postmortem examination—first, general considerations, followed by the special technics necessary to successful and adequate examination of each body system. A well written chapter on the examination of the skull and brain of the infant should appeal to pathologists in training and to those unfamiliar with the modifications in technic which are necessary to demonstrate hemorrhage and dural tears without the introduction of artefact produced by the examiner. The method of opening the heart so that cardiac defects will not be missed is also well presented. Chapter III is a survey of the principal causes of fetal and neonatal death, and deals with malformations, anoxia, birth trauma, toxemia and infections, in a general manner, pointing out the special anatomic features of each with comparisons of later life where indicated. Chapter IV continues the discussion of causes of fetal and neonatal death under the title "Special Pathology". In this, the pathology of the organic systems of the body are reviewed in greater detail. Included in this chapter is a short discussion of the Rh factor and its effects on the fetus and the newborn infant, attention being given also to the hemorrhagic diseases. Chapter V presents the statistical data pertaining to births, maternal deaths, infant deaths and stillbirths. It includes many tables and charts as a visual aid to those interested in a statistical study of the problem.

The book is clearly written. The illustrations and tables aid in clarifying the special features of morbidity and mortality of the fetus and the newborn infant. It is based chiefly on Dr Potter's vast experiences at the Chicago Lying-In Hospital and elsewhere in the city of Chicago. It is complete in its subject matter, although brief in detailed descriptions of the anatomic pathology. The reviewer has derived considerable satisfaction and information from this little book, and has found it a valuable guide in the teaching of residents. If there were a tendency to be critical, it would be only the wish that some day Dr Potter might find it desirable to do an exhaustive work on the special pathology of the fetal and newborn period of life.

METHODEN DER PATHOLOGISCHEN HISTOLOGIE By Frederic Roulet, A. O. professor of pathologic anatomy, prosector of the pathologic institute of the University of Basel. Pp 567, with 20 illustrations. Price, 15.40 dollars br bound. Vienna, Austria. Springer-Verlag, 1948.

For a generation German pathologists had been taught, histologic methods according to the classic "Pathologisch-histologischen Untersuchungsmethoden" of Schmorl. In those days "Schmorl" was found in every laboratory. Roulet's book, the reviewer believes, is destined to become "Schmorl's" successor. In this scholarly manual Roulet presents the material with special regard for the needs of the inexperienced student. He is detailed in his explanations and generous with

his references, a paragraph of citations of the more important relevant literature is added to each chapter. The technical methods described include the recent advances in the field of histochemistry and fluorescent microscopy. Phase-contrast microscopy is also discussed. An interesting chapter, and important for those pathologists who "review" histologic slides for diagnosis, is that which deals with the restaining of finished slides and the removing from such slides of certain impurities. Attention is paid to means of transporting material to distant places for histologic examination. There is also an extensive chapter on the demonstration of bacteria, protozoa and certain elementary bodies. Any one interested in histologic technic will find this manual an excellent reference book for procedures ranging from the simplest to the most elaborate specific determinations.

HOW TO BECOME A DOCTOR. A COMPLETE GUIDE TO THE STUDY OF MEDICINE, DENTISTRY, PHARMACY, VETERINARIAN MEDICINE, OCCUPATIONAL THERAPY, CHIROPODY AND FOOT SURGERY, OPTOMETRY, HOSPITAL ADMINISTRATION, MEDICAL ILLUSTRATION, AND THE SCIENCES. By George R. Moon, M.A., examiner and recorder, University of Illinois Colleges of Medicine, Dentistry and Pharmacy. Pp 131. Price \$2. Philadelphia and Toronto, Canada. The Blakiston Company, 1949.

PATHOLOGIE DES KOHLEHYDRATSTOFFWECHSELS. By Prof. Dr. E. Frank, director of the II. medizinischen Klinik der Universität Istanbul. Cloth. Pp 342, with 20 illustrations. Price 24 Swiss francs. Basel, Switzerland. Benno Schwabe & Co. Verlag. New York. Grune & Stratton, Inc., 1949.

This volume is a thoughtful consideration and an attempted synthesis of the physiologic, biochemical and clinical aspects of the various disturbances of carbohydrate metabolism. The author is an experienced older clinician, whose interest in the pathologic physiology of diabetes and other disturbances spans a considerable period of time. Nevertheless, it may be said to his credit that his outlook has advanced with the times and that his point of view, though conservative, is well balanced. Although written by a clinician, the book is not primarily clinical in nature. It will therefore be of greater interest to the physiologist and to the specialist in diabetes than to the general student of internal medicine.

EXPERIMENTAL IMMUNOCHEMISTRY. By Elvin A. Kabat, Ph.D., associate professor of bacteriology, College of Physicians and Surgeons, Columbia University and the Neurological Institute, New York, and Manfred M. Mayer, Ph.D., associate professor of bacteriology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore. With a foreword by Michael Heidelberger, Ph.D., professor of biochemistry, College of Physicians and Surgeons, Columbia University, and chemist to the Presbyterian Hospital, New York. Pp 567, with 88 illustrations. Price \$8.75. Springfield, Ill. Charles C. Thomas, Publisher, 1948.

This useful book, which is less than a text but more than an abridged compilation, may be described as a somewhat elaborated compendium of some principles and technics that are being used in the progress of immunology toward the status of a "science." Applicable principles and methods have been harvested from the fields of physics, organic, biologic and analytic chemistry, and even from mathematics. But, although mathematics should be applied, there are, and will be for a while, too much relativity and too many simplifications to ask the "Queen of the Sciences" to certify an authentic exactitude to many features of the antigen-antibody reaction.

This book details the conventional immunologic methods and includes descriptions of modern (current) devices for quantitative measurements. The number of special procedures is too long to list here, but in addition to the Kjeldahl, van Slyke and Folin-Ciocalteu methods (to which nominal list the Heidelberger-Kendall-MacPherson method should be added) the authors discuss unnamed methods involving electrophoretic and ultra-centrifugal analyses, diffusion, optical rotation, solubility and ultraviolet ray absorption spectrums.

The section on "preparations" has eighteen chapters, each dealing with purification of natural antigens or with couplings that involve azo-, acetyl-, malonyl-, phosphoryl-, phenylureids-, carbobenzoxy- and other acid-chloride linkages with antigens.

In a short appendix the mechanics of centrifugation, colorimetry, calibration, preparations of antigens and injection into animals are considered.

The "preview," a humorous, flavorful and penetrating foreword by Heidelberger—who quite unconvincingly denies paternity of this volume—reemphasizes the still too widely unrealized usefulness of immunochemical methodology for discriminatively qualitative and delicately quantitative study of the externally structural (and dependent) properties of compounds ranging from the fairly simple to the most highly complex.

This making of a book by two intimately experienced, alert, critical and vigorous young men merits high commendation.

INCIDENCE OF FATAL CORONARY DISEASE IN NONDIABETIC AND IN DIABETIC PERSONS

B J CLAWSON, M D

AND

E T BELL, M D

MINNEAPOLIS

THERE is a widespread impression that persons suffering from diabetes are more prone to have coronary disease than nondiabetic persons of corresponding age, but to our knowledge no extensive statistics supporting this impression have ever been published. We have therefore analyzed all of the postmortem records on file in the department of pathology of the University of Minnesota covering the period from 1910 through 1947. The number of postmortem examinations performed annually during this period increased from about 200 in 1910 to nearly 2,700 in 1947. The total number is 50,775. There were 49,593 nondiabetic and 1,182 diabetic subjects.

In the nondiabetic group there were 31,975 males and 17,618 females. The age distribution is shown in tables 1 and 2. Only those subjects of autopsies are listed as dying of coronary disease in whom both the clinical and the anatomic evidence indicated coronary disease as the major lesion. There were nearly as many with moderately severe coronary disease in whom death was due to some other cause, but these were not included.

In the nondiabetic males (table 1) it will be noted that there were no deaths from coronary disease in the first two decades of life and only 8 in the third. After the fifth decade the percentage of those whose deaths result from coronary disease does not increase with advancing age but remains about 10 per cent. In the nondiabetic females the incidence of deaths due to coronary disease is notably less than the incidence in males until the age of 70 years, after which it is about equal to that in the males. Among nondiabetic subjects of autopsies the ratio of males to females dying of coronary disease is about 5 to 3.

From the Department of Pathology, University of Minnesota

This investigation was aided by a grant from the Bureau of Naval Research

* It is shown in tables 1 and 2 that fatal coronary disease is about twice as frequent in diabetic as in nondiabetic males and three times as frequent in diabetic as in nondiabetic females. Among diabetic

TABLE 1—*Coronary Disease in Nondiabetic and in Diabetic Males Examined Post Mortem*

Decade, Years	Non diabetic Males	Coronary Disease		Diabetic Males	Coronary Disease	
		Number	Per Cent		Number	Per Cent
0 10	4,286	0	0	7	0	0
10 20	932	0	0	9	0	0
20 30	1,809	8	0.4	11	0	0
30 40	2,799	60	2.1	33	3	9.1
40 50	4,453	336	7.5	46	8	17.4
50 60	5,824	606	10.4	116	13	11.2
60 70	5,883	654	11.1	188	46	24.5
70 80	4,406	472	10.7	115	24	20.9
80 100	1,583	161	10.2	33	6	18.2
40 100	22,149	2,229	10	498	97	19.5

TABLE 2—*Coronary Disease in Nondiabetic and in Diabetic Females Examined Post Mortem*

Decade, Years	Non diabetic Females	Coronary Disease		Diabetic Females	Coronary Disease	
		Number	Per Cent		Number	Per Cent
0 10	3,121	0	0	2	0	0
10 20	799	2	0.25	12	0	0
20-30	1,584	0	0	28	0	0
30 40	1,843	4	0.2	36	1	3
40-50	2,158	19	0.9	51	5	10
50-60	2,469	90	3.6	134	17	12.7
60 70	2,525	178	7.0	199	34	17.1
70 80	2,205	225	10.2	129	33	25.6
80 100	914	88	9.5	33	6	18
40 80	10,271	600	5.8	546	95	17.4

persons the incidence of fatal coronary disease is almost as high in females as in males.

Diabetes therefore accelerates atherosclerosis of the coronary arteries, and it has a more pronounced effect in females than in males. About 4 per cent of deaths due to coronary disease in males and nearly 14 per cent in females are associated with diabetes.

PRIMARY ALVEOLAR CELL TUMORS OF THE LUNG

T C LAIPPLY, M D

AND

C I FISHER, M D

CHICAGO

TUMORS considered to arise from cells of the pulmonary alveoli are distinctly uncommon. On the basis of morphologic features such tumors may be divided into cancerous and noncancerous and into nodular and diffuse types. The noncancerous ones are usually referred to as pulmonary adenomatosis, the cancers are listed under the term "alveolar cell carcinoma."

The first report of bilateral pulmonary alveolar cell adenomatosis in man was that of Helly¹ in 1907. Since that time 14 other acceptable instances have been reported. In 6 of the 15 cases the tumor was diffusely distributed, being unilateral in 2 and bilateral in 3. In the other 9 cases the tumor was of the diffuse type, involving all lobes of both lungs.

The multiple nodular type of alveolar cell carcinoma was first described by Malassez³ in 1876. The diffuse type was first reported by Musser⁴ in 1903. Since these cases were recorded, 26 other instances of alveolar cell carcinoma have been reported.⁵ These, along

From the Departments of Pathology of Northwestern University and Wesley Memorial Hospital

1 Helly, K. *Ztschr f Heilk* **28** 105, 1907

2 (a) Lohlein, M. *Verhandl d deutsch path Gesellsch* **12** 111, 1908 (b) Gorden, A. K. *Lancet* **2** 501, 1920 (c) Reuss, H. *Ueber Zwei Falle multicentrisch entstandener Lungenkrebs*, Inaug Dissert, Hamburg, 1934 (d) Weissmann, S. *Frankfurt Ztschr f Path* **47** 534, 1935 (e) Bonne, C. *Am J Cancer* **35** 491, 1939 (f) Richardson, G. O. *J Path & Bact* **51** 297, 1940 (g) Sims, J. L. *Arch Int Med* **71** 403, 1943 (h) Bell, E. T. *Am J Path* **19** 901, 1943 (i) Taft, E. B., and Nickerson, D. A. *ibid* **20** 395, 1944 (2 cases) (j) Geever, E. F., Carter, H. R., Neubueger, K. T., and Schmidt, E. A. *Radiology* **44** 319, 1945 (k) Wood, D. A., and Pierson, P. H. *Am Rev Tuberc* **51** 205, 1945 (l) Simon, M. A. *Am J Path* **23** 413, 1947 (m) Drymalski, G. W., Thompson, J. R., and Sweany, H. C. *Am J Path* **24** 1083, 1948 (2 cases)

3 Malassez, L. *Arch de physiol norm et path* **3** 353, 1876

4 Musser, J. H. *Univ Pennsylvania M Bull* **16** 289, 1903

5 (a) Finlay, D., and Parker, R. W. *Lancet* **1** 838, 1877 (b) Kretschmer, W. H. *Ueber das primäre Bronchial- und Lungencarcinom*, Inaug Dissert, Leipzig, B. Georgi, 1904 (c) Pepere, A. *Centralbl f Path* **15** 948, 1904, cited

(Footnote continued on next page)

with the 2 cases reported in this paper, make a total of 30 instances of alveolar cell carcinoma of the lung. In all 30 cases the tumor involved both lungs. In 11 of these it was of the multiple nodular type, in 10 it was of the diffuse type, and in 9 there were both nodular and diffuse types of neoplasm in different parts of the lungs. Some of these cancerous growths produced metastases, others were invasive. In 25 cases there were extrapulmonary metastases, in 3 there was tumor within pulmonary vessels, and in 2 there was local invasion of interstitial tissue and pleura.

The number of cases of primary alveolar cell tumor accepted as authentic and their classification as "benign" or "malignant" vary in different reports. The evaluation of recorded cases is difficult because of incompleteness of microscopic descriptions, failure to exclude other organs as primary sites of tumor and the unwillingness of many authors to admit the alveolar cell origin of such tumors. As indicated by Neubuerger and Geever⁶ in their review of this subject, there are many controversial and not completely acceptable cases that in all probability belong to cancerous and noncancerous groups. The morphologic features of pulmonary adenomatosis and alveolar cell carcinoma are so similar that it seems highly probable that they are related. Nevertheless, most of the reports deal either with pulmonary adenomatosis or with alveolar cell carcinoma, and in only a few, like those of Neubuerger and Geever⁶ and Simon,²¹ is the possibility of a relationship between the two conditions suggested. Both pulmonary adenomatosis and alveolar cell carcinoma occur in two distinct forms, *i. e.*, multiple nodular and diffuse. As already indicated, both conditions are usually bilateral and involve all lobes of both lungs. The diffuse type of lesion has a gross appearance like that of lobar pneumonia in the stage of gray hepatization. Mucin may be produced in sufficiently

by Neubuerger and Geever⁶ (d) Knerim, H. *Verhandl. d. deutsch. path. Gesellsch.* **13**: 407, 1909. (e) Briese. *Frankfurt Ztschr. f. Path.* **23**: 48, 1920. (f) Schmincke, A. *Centralbl. f. Path.* **33**: 18, 1922, cited by Neubuerger and Geever⁶. (g) Godel, A. *Frankfurt Ztschr. f. Path.* **29**: 375, 1923. (h) Hedinger, E. *Schweiz. med. Wchnschr.* **53**: 165, 1923. (i) Rusk, G. Y., and Randolph, V. *J. A. M. A.* **82**: 442, 1924. (j) Fried, B. M. *Arch. Int. Med.* **35**: 1, 1925. (k) Oberndorfer, S. *Virchows Arch. f. path. Anat.* **275**: 728, 1930. (l) Sweany, H. C. *Arch. Path.* **19**: 203, 1935. (m) Graber, K. *Beitrag zur Histopathologie des primären Lungenkrebses*, Inaug. Dissert., Munich, Speyer, Pilger, 1938, abstracted, *Centralbl. f. Path.* **77**: 93, 1941. (n) Casilli, A. R., and White, H. J. *Am. J. Clin. Path.* **10**: 623, 1940. (o) Neubuerger, K. T. *J. Thoracic Surg.* **10**: 557, 1941. (p) Dacie, J. V., and Hoyle, C. *Brit. J. Tuberc.* **36**: 158, 1942. (q) Wood, E. H. *Radiology* **40**: 193, 1943. (r) Geever and others²¹ (5 cases). (s) Drymalski and others^{2m}.

6 Neubuerger, K. T., and Geever, E. F. *Arch. Path.* **33**: 551, 1942.

large amounts to be recognized grossly as a viscid translucent material in the affected portions of the lung, thus the gross picture may be confused with that of pneumonia caused by Friedlander's bacillus or *Pneumococcus* type III. The striking microscopic features of pulmonary adenomatosis are alveoli lined with epithelium-like cells, marked fibrosis and lymphocytic infiltration of interalveolar septums (fig 1). The lining alveolar cells are columnar or cuboidal and usually non-ciliated (fig 2). They are uniform in size, shape and staining reaction. Mitotic figures are few, and there is no invasion of adjacent tissue. The nuclei are usually basally situated and homogeneous in appearance. The cytoplasm is eosinophilic and may be vacuolated. In some instances the cells are of goblet type. Mucin may be present within the lining alveolar cells and within the alveolar spaces (figs 1 and 2). In some instances, however, mucin has not been satisfactorily demonstrated. The microscopic characteristics of alveolar cell carcinoma (figs 5 and 6) are similar to those of adenomatosis—cellular pleomorphism, invasion and metastases being the contrasting differences. It should be noted that in the cases reported by Dacie and Hoyle^{5p} and Wood,^{5q} as well as in case 1 of this paper, the changes in all lobes of the lungs are like those of pulmonary adenomatosis except for small foci in a lobe of one lung, where there is demonstrable invasive carcinoma, these cases are considered to be examples of diffuse pulmonary adenomatosis with focal cancerous change. In 2 of the other cases classed as instances of cancerous pulmonary adenomatosis there was vascular invasion, and in the remaining 25 cases there were extrapulmonary metastases. It thus seems possible that pulmonary adenomatosis in man may be an early stage of alveolar carcinoma or that it may undergo cancerous change. The differentiation of alveolar cell adenomatosis and alveolar cell carcinoma on the basis of the cytologic aspects alone may be difficult (figs 2 and 5). This is exemplified in the cases reported by Briese^{5e} and Oberndorfer^{5k}. In both instances the cases have been unjustifiably classed as instances of pulmonary adenomatosis in spite of the existence of extrapulmonary metastases.

The controversial question regarding the origin and the nature of alveolar lining cells still exists. Many authors, such as Bensley and Bensley,⁷ Miller,⁸ Bremer⁹ and Cooper,¹⁰ have maintained that the alveoli are lined by a continuous epithelial layer. Others, including

7 Bensley, R. D., and Bensley, S. H. *Anat. Rec.* **64**: 41, 1935.

8 Miller, W. S. *The Lung*, Springfield, Ill., Charles C. Thomas, Publisher, 1937.

9 Bremer, J. L. *Contrib. Embryol.* **25**: 83, 1935.

10 Cooper, E. R. A. *J. Path. & Bact.* **47**: 105, 1938.

Maximow and Bloom,¹¹ Lang,¹² Rose,¹³ Fried¹⁴ and Loosli,¹⁵ have considered the lining alveolar cells to be mesenchymal. Still others, like Ross,¹⁶ have expressed the belief that both mesenchymal and epithelial cells line the alveoli, and Bell^{2b} indicated that occasional epithelial cells may be found in postnatal lungs. This problem has still to be definitely settled, but in view of the work of Oppenheimer¹⁷ suggesting the possibility of mesenchymal cells giving rise to epithelium-like cellular elements it may be less important than was formerly thought. In none of the acceptable reported cases of alveolar cell tumor of human beings can a bronchial focus be demonstrated. In many instances the tumor appears to be multicentric in origin and to arise directly from alveolar walls. The tumor cells are usually not ciliated and are not continuous with bronchiolar epithelium. The morphologic features of the tumor cells and the fact that they produce mucin indicate that they are of epithelial type. At the present time it cannot be stated with certainty whether these cells originate from preexisting epithelial cells or as a result of metaplasia of an indifferent type of mesenchymal cell. The fact that in case 1 some of the cells lining alveoli have distinct cilia (fig 2) could be used to support the contention that such cells, at least in this instance, are bronchiolar or bronchial in origin.

These tumors of human beings are also of interest because of certain similarities particularly of the "benign" type to a pulmonary lesion of sheep,¹⁸ mice,¹⁹ horses²⁰ and guinea pigs²¹. That the condition occurs in sheep has been known since about 1891. It has been referred to as jagziekte, epizootic adenomatosis, pulmonary adenomatosis and infectious adenomatosis. Very similar and probably identical conditions have been reported under the titles of verminous pneumonia^{22a} and Montana progressive pneumonia²³ of sheep. The disease of sheep has

11 Maximow, A., and Bloom, W. A. *A Textbook of Histology*, Philadelphia, W. B. Saunders Company, 1930.

12 Lang, F. J. *J. Infect. Dis.* **37**: 430, 1935.

13 Rose, S. B. *Arch. Path.* **6**: 36, 1928.

14 Fried, B. M. *Medicine* **10**: 373, 1931.

15 Loosli, C. G. *Am. J. Anat.* **62**: 375, 1937.

16 Ross, I. S. *Arch. Path.* **27**: 478, 1939.

17 Oppenheimer, J. *Quart. Rev. Biol.* **15**: 1, 1940.

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been reported in widely scattered parts of the world, such as South Africa, England, Iceland, Germany and Montana. In such localities it has been responsible for marked economic losses, some farms having lost 50 to 85 per cent of their flocks because of this disease. The name *jagziekte* is derived from the Dutch words *jagt*, to drive, and *ziekte*, sickness, and is intended to indicate that the first symptoms appear in animals that are fatigued as a result of having been driven a long distance. The exact cause of the disease is unknown, but it is commonly believed to be infectious and capable of epidemic spread. Many workers hold that it is of viral origin, which is supported but not completely established by the work of Dungal.^{22b} Neither the possibility of its being a nonspecific response to irritants as suggested by Grumbach²¹ nor the idea of De Kock²⁴ that it is a true neoplasm can be excluded. The latter view is supported by Aynaud's²⁵ report of an instance in which pulmonary adenomatosis of a sheep had metastasized to regional lymph nodes.

The gross and microscopic features of the disease of sheep are similar to those noted in human cases of "benign" alveolar cell tumors. Thus, in sheep the lesions are usually extensive in both lungs, the normal pulmonary structure is retained, lining alveolar cells are of cuboidal or columnar type, proliferation and lymphocytic infiltration of interstitial tissue are impressive, and, with the exception of the case reported by Aynaud,²⁵ invasion and metastases are absent. There are usually minor differences in the appearance of the epithelial cells, but the morphologic similarities are striking. Nevertheless, at present the belief that there is a significant relation between the disease of sheep and the alveolar cell tumors of human beings is speculative and not clearly established.

REPORT OF CASES

CASE 1—L. W., a white woman aged 51 years, was admitted to Wesley Memorial Hospital first on July 10, 1947 with complaints of difficulty of breathing, cough and weakness. Her breathing had become progressively labored since one year before, at the time of hospitalization climbing one flight of stairs produced marked dyspnea and discomfort. The cough began three months before admission, it was nonproductive and frequently was nocturnal and paroxysmal, so that she could not sleep. There was no history of hemoptysis, cyanosis, pain of the chest or orthopnea. The patient had lost approximately 15 pounds (7 Kg.) in weight during the previous three months. She also complained of loss of appetite, diarrhea and weakness of a few weeks' duration. On admission her temperature was 98 F., pulse rate, 80, respiratory rate, 34, blood pressure, 120 systolic and 80 diastolic. She was cyanotic and breathing rapidly, with effort, the veins of the neck distended.

²⁴ De Kock, G., in Annual Report of the Director of Veterinary Service, Department of Agriculture Union of South Africa, 1929, vol 2, sect 1-18, pp 611 and 1169-1183.

²⁵ Aynaud, M. Compt rend Soc de biol 95 1540, 1926.

There was a male distribution of hair over the chest, abdomen and pubis, the breasts were normal. The left border of the heart was in the fifth intercostal space and midclavicular line, with no murmurs or thrills, gallop rhythm was noted. The lungs showed diminished resonance to percussion, and loud inspiratory rales were heard over the lower lobes posteriorly. In the abdomen the liver was palpable 2 cm below the right costal margin, there were no masses or tenderness. The nail beds of the extremities were cyanotic, there was 2 plus pitting edema of the lower legs. Laboratory examination showed 300 mg of albumin and 1 to 3 white blood cells in the urine, 4,030,000 erythrocytes and 9,200 white cells per cubic millimeter of blood, 14 Gm of hemoglobin per hundred grams of blood, a negative Wassermann reaction of the blood, an erythrocyte sedimentation rate of 18 mm per hour, nonprotein nitrogen 55.6 mg, albumin 2.56 Gm and globulin 3.57 Gm per hundred cubic centimeters of blood, an electrocardiographic pattern characteristic of right-sided heart strain, roentgen evidence of a 15 per cent enlargement of the cardiac silhouette and slight passive congestion of the lungs.

The patient was placed at rest in bed and treated with nasally administered oxygen, digitoxin and ammonium chloride. Her symptoms and signs became less marked, and her nonprotein nitrogen was 35.5 mg per hundred cubic centimeters on the nineteenth hospital day. Slight dyspnea and cyanosis persisted, and she was discharged on the forty-third hospital day.

The patient was readmitted to the hospital on Sept 25, 1947. At this time she complained of marked difficulty in breathing, of gaining 30 pounds (13.5 Kg) in four weeks and of "shingles" of the left side of the chest. Her temperature was 97.8 F, pulse rate, 96, respiratory rate, 40, blood pressure, 110 systolic and 90 diastolic. There were marked cyanosis, dyspnea and orthopnea. The left border of the heart was in the anterior axillary line, the pulmonic second sound was accentuated, being much louder than the aortic second sound. The lungs had coarse moist rales throughout the lower lobes. In the abdomen the liver was palpable 1½ fingerbreadths below the right costal margin, there was ascites of moderate degree. The lower extremities showed 4 plus pitting edema extending up to the middle of the thighs. Laboratory examination showed 300 mg of albumin in the urine, red blood cells, 4,900,000, with 15.5 Gm of hemoglobin, white blood cells, 16,500, 91 per cent of which were neutrophilic granulocytes and 9 per cent lymphocytes, chlorides 413 mg, nonprotein nitrogen 41.7 mg, albumin 2.31 Gm and globulin 4.23 Gm per hundred cubic centimeters of blood.

The patient's condition became progressively worse, and she died on her third hospital day. The clinical diagnosis was Ayerza's disease.

Autopsy (C. I. Fisher, M.D.) revealed hypertrophy and dilatation of the right atrium and the right ventricle, marked sclerosis of pulmonary arteries and arterioles, bilateral acute fibrinous pleuritis, bilateral acute bronchopneumonia, diffuse alveolar cell tumor involving both lungs and invading the interstitial tissue and the visceral pleura of the lower lobe of the right lung, herpes zoster of the skin of the left side of the thorax, moderate arterial and arteriolar nephrosclerosis, ascites (1,400 cc), moderate edema of the lower extremities.

The heart weighed 305 Gm. The hypertrophy was limited to the right atrium and the right ventricle. The wall of the right ventricle, in spite of marked dilatation of the chamber, measured 8 mm in thickness.

The right and left lungs weighed 800 and 600 Gm, respectively. All lobes of the lungs were increased in size and showed uniformly increased consistence. Crepitation was greatly reduced. The cut surfaces were homogeneous and pale gray mottled with black. In the peripheral and posterior portion of the lower lobe

of the right lung there were two spherical, firm, pale yellowish gray nodules with maximum measurements of 0.9 and 2.0 cm. These nodules had no demonstrable connection with bronchi. Numerous microscopic sections of all lobes of the lungs revealed an impressive increase in the amount of interstitial connective tissue, in which there were many lymphocytes and plasma cells (fig 1). The alveoli were lined with ciliated high columnar cells of epithelial type (fig 2). Some of these cells, as well as the alveoli, contained material which stained positively for mucin by the mucicarmine method. The two nodules in the lower lobe of the right lung were made up of richly cellular, pleomorphic, invasive neoplasm that formed glands and mucin (figs 3 and 4). Throughout both lungs the microscopic features were like those of pulmonary adenomatosis, and in the circumscribed nodules in the lower lobe of the right lung they were those of invasive adenocarcinoma. No metastatic tumor was demonstrated in either regional lymph nodes or other viscera. A complete autopsy was performed and no other site of primary tumor was demonstrated.

Sclerosis was slight in the large pulmonary arteries but striking in the small pulmonary arteries and arterioles. Many of the sclerotic vessels showed intense lymphocytic infiltration, particularly of the thickened intimal portions.

CASE 2—A. D., a 45 year old white man, was admitted to Wesley Memorial Hospital on May 4, 1948. His chief complaint was pain in the chest on coughing, of nine months' duration. During the month preceding hospitalization he had noted hemoptysis, shortness of breath, loss of appetite and an increase in the severity of the thoracic pain. For a few days before admission the pain radiated from the back to the front of the chest on the left side and was brought on by deep inspiration or sudden movement of his back. During the course of his present illness he had been treated for "arthritis of the spine." He estimated that he had lost 25 pounds (11 Kg.) since the onset of his illness. On the evening prior to admission, after an attack of coughing, he noted severe pain in the left dorsal region, which persisted and prevented him from sleeping. His past history was non-contributory. On admission his height was 5 feet 9 inches (175 cm.), weight, 140 pounds (63.5 Kg.), temperature, 100 F., pulse rate, 156, respiratory rate, 44, blood pressure 120 systolic and 80 diastolic. In appearance he was well developed, moderately undernourished, pale, slightly cyanotic and evidently in pain. In the neck, in the supraclavicular region, bilaterally, there were several firm, discrete, nontender lymph nodes measuring 1 to 2.5 cm. in maximum measurement. The heart was not enlarged to percussion, and there were no murmurs or abnormal rhythm. The thorax was symmetric. Its expansion was markedly limited, and there were tenderness posterolaterally on the left, dullness at the bases of the lungs posteriorly, bronchial breathing and coarse rales laterally at the base of the left lung. Back motion was limited because of pain in the thoracic region. The remainder of the examination revealed nothing of significance.

At admission the urine was normal. The blood showed 5,400,000 erythrocytes and 30,500 white cells (92 per cent neutrophilic granulocytes, 8 per cent lymphocytes), the hemoglobin content was 16 Gm., the hematocrit reading 46, a blood culture was negative, the blood nonprotein nitrogen was 40.3 mg. per hundred cubic centimeters. Seven examinations of sputum revealed no acid-fast bacilli. Roentgen examination showed 30 per cent enlargement of the cardiac silhouette, mediastinal enlargement, probably due to lymphadenopathy, diffuse mottling due to small areas of increased density in all lobes of the lungs particularly in the central region and in the lower lobes, slight atelectasis of the middle lobe of the right lung, the left leaf of the diaphragm slightly higher than the right.

Three days after hospitalization the white blood cell count was 9,950, with 88 per cent neutrophils and 12 per cent lymphocytes. For thirteen days he showed symptomatic improvement, becoming less cyanotic and less dyspneic. A roentgenogram of the chest at this time showed no noteworthy change. On his

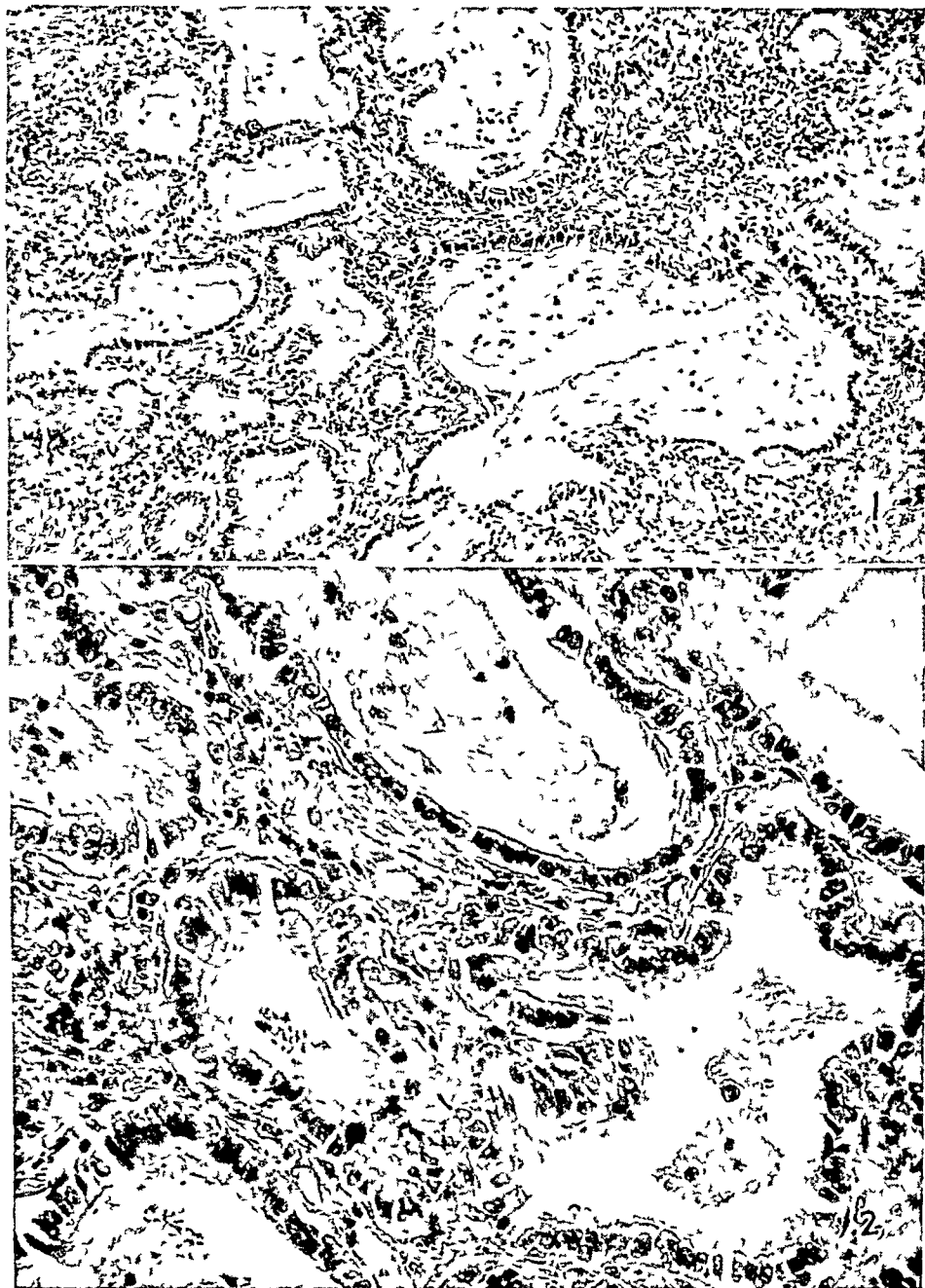


Fig 1 (case 1)—Alveoli of the upper lobe of the left lung, $\times 290$. They contain mucin and are lined with high columnar cells. The interstitial tissue is increased in amount and infiltrated by lymphocytes.

Fig 2 (case 1)—Alveoli of the upper lobe of the left lung, $\times 550$. They are lined with high columnar cells of mucin-secreting type. No noteworthy cellular pleomorphism is evident. A few of the cells have identifiable cilia.

fourteenth hospital day he noted severe pain in the left side of his chest with marked dyspnea, cyanosis and tachycardia. Two days later, shortly after precordial pain, cyanosis and pink sputum had been noted, gasping respirations developed and the patient died.

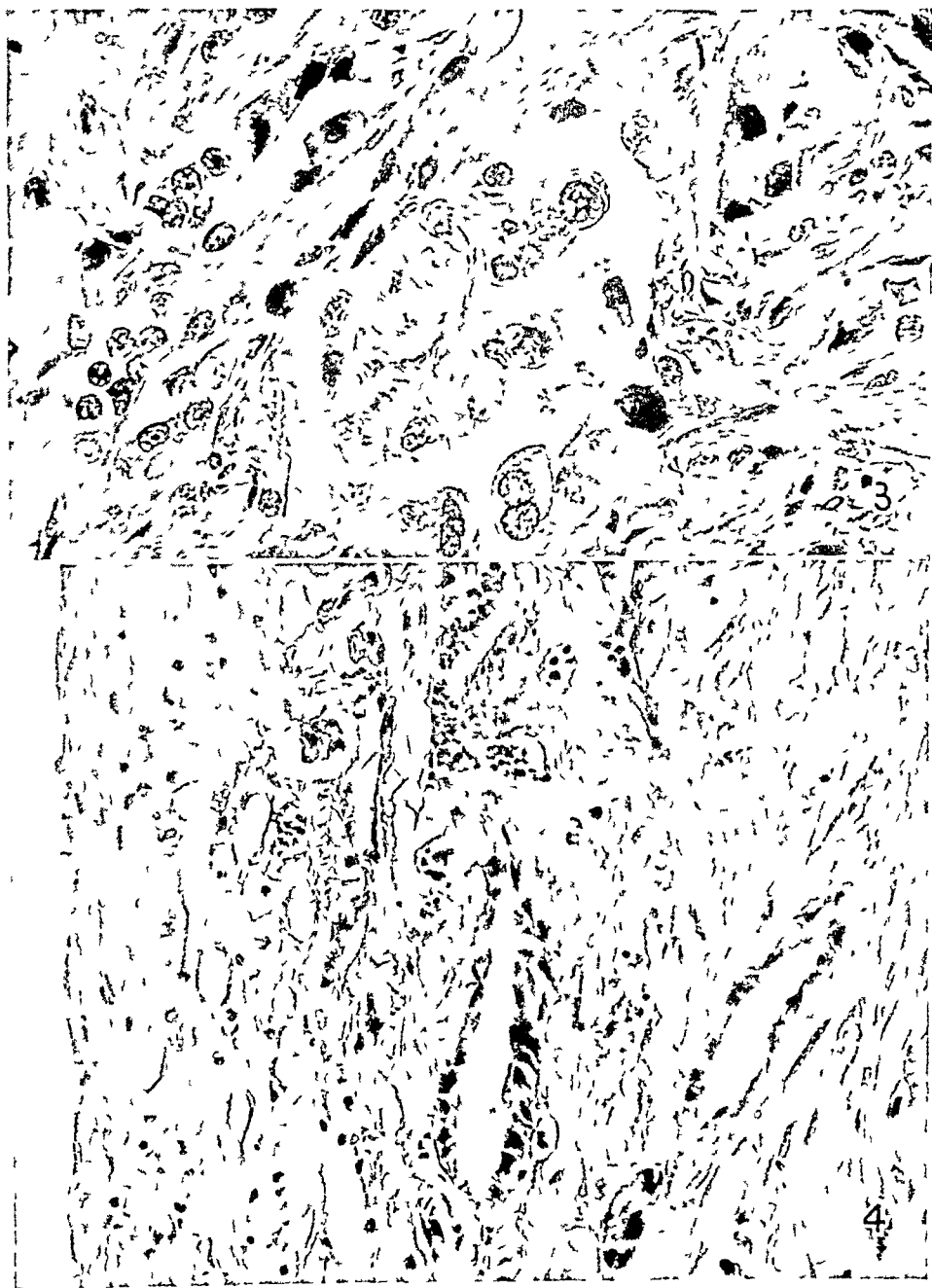


Fig 3 (case 1) —Glands formed by a tumor nodule of the lower lobe of the right lung, $\times 690$. They are lined with atypical cells, abnormal nuclei and mitoses are evident.

Fig 4 (case 1) —Neoplasm invading visceral pleura of the lower lobe of the right lung, $\times 500$.

Autopsy (Dr F A Svec) revealed primary alveolar cell carcinoma of the lungs with invasion of pleura and intrapulmonary lymphatics and metastases in mediastinal and cervical lymph nodes and the left adrenal gland, recent organizing thrombosis of peripheral branches of the pulmonary artery to the upper and lower lobes of the left lung, organizing infarcts in the upper and lower lobes



Fig 5 (case 2) —Alveoli of the lower lobe of the left lung, $\times 500$ Columnar cells without marked pleomorphism line the alveoli

Fig 6 (case 2) —Alveoli of the upper lobe of the right lung, $\times 65$ They are lined with high columnar cells Papillae of tumor project into alveolar spaces

of the left lung, hydropericardium (500 cc), hydrothorax of the left side (1,500 cc), hypertrophy and dilatation of the heart, particularly of the right

atrium and the right ventricle (400 Gm), marked passive hyperemia of viscera, slight arterial and arteriolar nephrosclerosis

The right and left lungs weighed 1,150 and 900 Gm, respectively. The increase in size and weight was due principally to the presence of numerous nodules, varying from 0.1 to 1.5 cm in maximum measurement. All the nodules were firm, pale yellow and sharply circumscribed from adjacent pulmonary tissue. They were diffusely distributed throughout all lobes of both lungs. None of the tumor nodules was outstanding in size, and bronchial involvement could not be demonstrated. Two pyramid-shaped, yellowish red infarcts were present in the left lung. The maximum measurement of the infarct in the upper lobe was 6 cm and of that in the lower lobe 7 cm. The branches of the left pulmonary artery that supplied these regions were filled with adherent, firm, friable, reddish yellow thrombi. Microscopic examination of many sections from all lobes of the lungs showed the nodules to be similar at all sites. They were made up of richly cellular tumor of epithelial and papilliferous type (fig 5). In many regions alveoli were lined with columnar tumor cells, which in most regions were of fairly regular type without definite variation in their size and staining properties. Papillae were evident in many regions (fig 6), and sections stained by the mucicarmine method revealed mucin within tumor cells and within alveolar spaces that were lined with such cells. A complete autopsy was performed and no other site of primary tumor was demonstrated. The tumor is considered to be the multiple nodular type of primary alveolar cell carcinoma.

The heart, which weighed 400 Gm, showed significant enlargement of the right atrium and the right ventricle. The hypertrophy and the dilatation of these cardiac chambers are considered to be secondary to increased resistance to blood flow and probably increased pressure within the pulmonary circulatory system which resulted from compression and obliteration of pulmonary vessels. The thrombi in branches of pulmonary arteries and the infarcts in the left lung could have been contributing factors in reducing the volume of the pulmonary circulatory system. The immediate cause of death is considered to have been cardiac failure.

COMMENT

The clinical signs and symptoms of primary alveolar cell tumors are usually not diagnostic. The symptoms include cough, hemoptysis, cyanosis, dyspnea, pain in the chest and evidence of pleural effusion. Lack of fever, loss of weight, progressive weakness, a chronic course and obvious dyspnea are sometimes suggestive of a pulmonary tumor. Nevertheless, the roentgen changes are not pathognomonic. In the majority of the reported cases the condition has been diagnosed as either pneumonia (occasionally of atypical type) or tuberculosis.

In case 1 the patient had signs and symptoms that led to a diagnosis of Ayerza's disease. Changes noted at autopsy, i.e., diffuse fibrosis of the lungs, marked sclerosis of pulmonary arteries and arterioles, hypertrophy and dilatation of the heart, are consistent with such a diagnosis. According to Brenner's²⁶ classification, this would be secondary pulmonary arteriosclerosis, which is the type that evidently existed in the patient described by Ayerza in 1901. The gross and

microscopic features of the lungs are like those of the diffuse type of pulmonary alveolar cell adenomatosis. The case differs from most recorded cases of this condition in that some of the cells lining alveoli had distinct cilia and in that there were foci of cancerous change. The two foci of invasive neoplasm in one lobe of the lung are considered to have originated from altered alveolar cells like those present in other parts of the lungs. It is thought, therefore, that this is an example of diffuse pulmonary alveolar cell adenomatosis with two foci of cancerous change. The case is classified as one of alveolar cell carcinoma.

It is worthy of note that a similar case was reported by Dacie and Hoyle.¹⁰ Diffuse pulmonary changes, one focus of "malignant" change and hypertrophy and dilatation of the right atrium and ventricle of the heart were noted. Another case in which focal cancerous change occurred was reported by Wood.⁵¹ These 2 cases, as well as our case 1, indicate that pulmonary alveolar cell adenomatosis may undergo cancerous change and support the view that pulmonary alveolar cell adenomatosis and alveolar cell carcinoma are related.

Our second case is one of the multiple nodular type of alveolar cell carcinoma. The cytologic aspect does not in itself clearly indicate that the lesion is cancerous. Nevertheless, the invasion of visceral pleura and of intrapulmonary lymphatics and the metastases in regional lymph nodes and in the left adrenal gland establish the cancerous nature of the tumor. In all probability there was partial obstruction and obliteration of many pulmonary vessels which led to increased resistance to blood flow and increased pressure within the pulmonary circulatory system. This in turn is considered to have caused the hypertrophy and dilatation of the right ventricle and ultimate cardiac failure.

SUMMARY

Two cases of pulmonary alveolar cell tumor of the lung are reported. One is considered to be a case of diffuse alveolar cell adenomatosis with two foci of cancerous change, the second is illustrative of bilateral multiple nodular alveolar cell carcinoma. In both instances the clinical signs and symptoms were those of chronic pulmonary disease with failure of the right side of the heart.

A review of the literature disclosed 45 acceptable cases of primary alveolar cell tumor. In 30 it had been established that the tumor was "malignant," and in 15, that it was "benign."

The striking similarity of morphologic features suggests but does not establish that there is a relationship between alveolar cell tumors of man and similar lesions in sheep, mice, horses and guinea pigs.

ARSENICAL ENCEPHALOPATHY

Report of a Case

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AND

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ACUTE hemorrhagic necrosis of the central nervous system following arsenical therapy has been described in the literature under the following terms "hemorrhagic encephalitis," "cerebral purpura," "toxic myelitis," "toxic myelopathy," "medullary perivascular necrosis," "pericapillary encephalorrhagia" and "serous apoplexy." The term "arsenical encephalopathy," suggested by several authors of recent papers on the subject, seems more appropriate.

Since 1903, when "hemorrhagic encephalitis" was first described by Rosenfeld,¹ many cases have been recorded in the clinical literature, but Globus and Ginsburg² in reviewing the literature up to 1933 were able to collect only 60 such cases in which the diagnosis was confirmed by necropsy. At least 23 additional cases have been recorded since that time, providing a total of 83 published cases.

REPORT OF CASE

A 34 year old white pharmacist had, in December 1946, a urethral discharge and a penile lesion shown by dark field examination to be syphilitic. He was given a course of 2,400,000 units of penicillin intramuscularly over seven and one-half days. On Jan 13, 1947, 0.3 Gm of neoarsphenamine was given intravenously without apparent untoward effects. One week later 0.45 Gm of neoarsphenamine was given intravenously. Forty-eight hours later he called the hospital and stated that he was aching all over, felt he was getting the "flu" and requested a prescription of codeine. At 2 p.m. of the same day his employer sent him home from work because he was listless and looked ill. The next morning the employer went to the patient's apartment and was unable to arouse him. A private physician found the patient disoriented, mentally confused and weak, with poor muscular coordination.

He was admitted to an out of town hospital, where examination revealed hyperactive reflexes, a bilateral Babinski sign, more marked on the right, and

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1 Rosenfeld, M. *Deutsche Ztschr. f. Nervenhe.* **24**: 415, 1903.

2 Globus, J. H., and Ginsburg, S. W. *Arch. Neurol. & Psychiat.* **30**: 1226, 1933.

marked tonic contraction of the right leg. Papilledema was present bilaterally. The cerebrospinal fluid contained 6 leukocytes per cubic millimeter and gave a negative reaction in the Pandy test, the pressure was 400 mm of water. The blood leukocyte count was normal. There was albuminuria (reaction for albumin, 1 plus). The temperature was normal and the pulse rate 60 per minute. The patient remained unresponsive and soon became incontinent of urine.

He was admitted to the Veterans Administration Hospital, January 25, seventy-two hours after onset, with a diagnosis of brain tumor. At this time he was entirely unresponsive even to painful stimuli. The body temperature was 102.2 F (rectal), pulse rate, 150, blood pressure, 120 systolic and 100 diastolic, respiratory rate, 40. The pupils were dilated and fixed, and the eyes appeared to drift to the left. Funduscopic findings were normal. There was a mucopurulent nasal discharge. Respirations were rapid and stertorous. Breath sounds were reduced at the bases of both lungs, and fine crepitant rales were heard over the left lateral chest wall. All extremities were flaccid. Tendon reflexes appeared normal in the upper extremities but were absent in the lower. Hoffmann's sign was not present. Babinski, Oppenheim, Chaddock and Gordon signs were present bilaterally.

On admission lumbar puncture revealed a pressure of 140 mm of water. The fluid contained 6 leukocytes per cubic millimeter, the protein content was 190 mg per hundred cubic centimeters, and serologic tests for syphilis (Kolmer and Kahn) were negative. The colloidal gold curve was 0112332000. Cultures were negative. The urine contained albumin (3 plus), 4 leukocytes and 10 to 15 erythrocytes per high power field, and was loaded with finely granular and hyaline casts. The blood leukocyte count was 12,700, with 86 per cent mature neutrophils, 12 per cent lymphocytes and 2 per cent monocytes. Blood urea nitrogen was 31 mg and blood sugar 102 mg per hundred cubic centimeters, and the blood serum Kahn and Kolmer tests were negative.

Urine and blood showed no significant alteration of the findings mentioned on repeated examination. A progressive drop in the spinal fluid protein with a significant rise in pressure was noted.

Temperature and pulse rate continued to rise, the temperature reaching a high of 107 F. Tendon reflexes disappeared, first on the left and a day later on the right. Penicillin was given intramuscularly, 30,000 units every four hours, and general supportive measures were initiated. On the third day of hospitalization a diagnosis of arsenical encephalopathy was made, and treatment with 2, 3-dimercaptopropanol (British anti-lewisite, or BAL) was begun, 7 cc every four hours, and continued until death. An electrocardiogram indicated myocardial damage. Death occurred at 3:45 a.m. on the fourth day, sixty-eight hours after admission, one hundred and forty hours after onset and one hundred and eighty-eight hours after the last injection of neoparsphenamine.

Postmortem Observations—There were bilateral confluent bronchopneumonia, mucosal hemorrhages of the stomach and the urinary bladder and slight capsular hemorrhage of the right adrenal gland. The large arteries showed moderate atheromatosis.

The weight of the fresh brain was 1,565 Gm. The gyri of both hemispheres showed slight flattening. Examination of frontal sections of the brain followed fixation in 4 per cent formaldehyde solution. At the level of the optic chiasm there were punctate hemorrhages in the white substance beneath the island of Reil, occurring in symmetric areas. A few scattered small hemorrhages were seen in the white substance of the frontal lobes.

In a section immediately anterior to the mamillary bodies there were hemorrhages in bilateral symmetric areas in the white substance beneath the island of Reil and bordering on the putamen. On the right side the center of the hemorrhagic area appeared necrotic. An area of closely spaced petechial hemorrhages was found on the left side of the corpus callosum. Isolated hemorrhages were seen throughout the white substance and extremely fine petechiae could be seen in the gray cortex of the right parietal lobe.

In a section immediately posterior to the mamillary bodies, symmetric areas of hemorrhagic necrosis were found on each side of the corpus callosum. Petechial hemorrhages were present in the ependyma of the inferior cornu of each lateral ventricle.

In a section through the nucleus ruber, symmetric hemorrhages and necrosis of the corpus callosum were seen similar to those found in the previous section. Small clumps of petechial hemorrhages were found in the lateral portion of each thalamus, and those on the right were the more prominent.

In a section through the pineal gland, symmetric hemorrhages of the corpus callosum and of the walls of the inferior cornu of each lateral ventricle were evident.

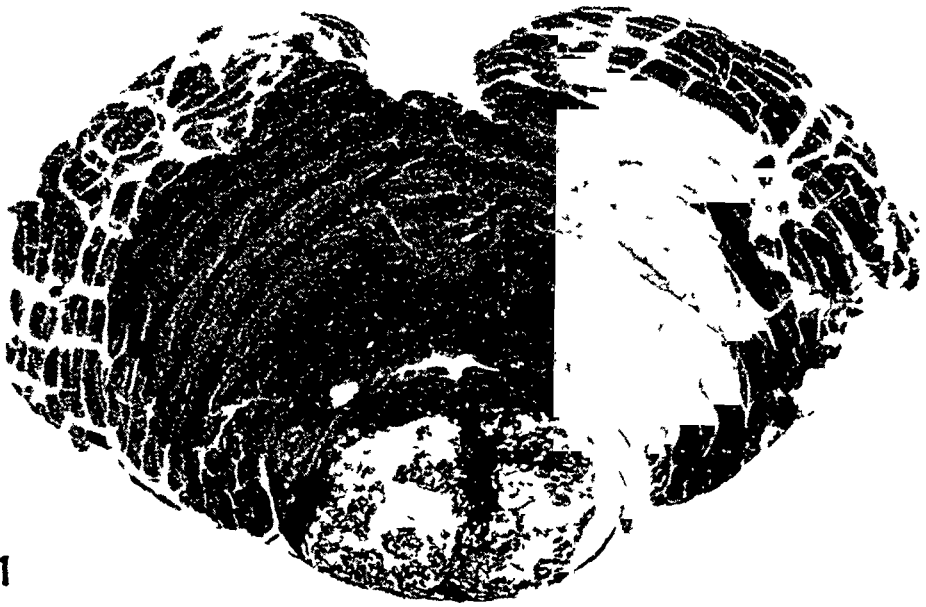
In a section through the posterior extremities of the lateral ventricles, an uneven zone of hemorrhage up to 1.0 cm. in width surrounded each lateral ventricle, and that on the right was slightly more prominent.

As to the brain stem and cerebellum, transverse sections through the upper one third of the pons showed bilateral symmetric hemorrhagic necrosis of the anterior two thirds (fig. 1). Necrosis and hemorrhage extended throughout the pons to its junction with the medulla oblongata, but not caudal to this. The medulla and the entire spinal cord showed dilated veins on the surface of the cord and a few perivascular hemorrhages in the medullary substance.

Microscopic Examination of Brain—Paraffin sections were stained by hematoxylin and eosin, Mallory phosphotungstic acid-hematoxylin and Van Gieson methods.

Medial Portion of Occipital Cortex, Including Lateral Ventricle The principal lesions were in the white substance around the wall of the lateral ventricle and consisted of advanced degeneration and poorly defined areas of necrosis in a broad irregular zone around the ventricle. All of the necrotic foci were surrounded by broad areas of interstitial hemorrhage. Most of the small vessels, including arterioles, capillaries and venules, were occluded by thrombi. The walls of many of the arterioles and capillaries appeared necrotic, but leukocytic infiltration was minimal. There was no appreciable degree of proliferation of the glia. Areas of degenerated brain substance around and between the hemorrhages were coarsely vacuolated. Evidence of degeneration of the cortical gray substance was slight. Perivascular edema was slight.

Clastrum The lesions consisted of a poorly defined area of necrosis and innumerable sharply defined perivascular hemorrhages in a broad zone around the area of necrosis (fig. 2). One or more necrotic small blood vessels were found within each circle of hemorrhage. Almost all of the arterioles, venules and capillaries appeared to be occluded by fibrinous thrombi, which appeared deep purple to black in the sections stained with phosphotungstic acid-hematoxylin. The erythrocytes were preserved. Small numbers of polymorphonuclear neutrophils infiltrated the necrotic tissue. The cortical gray substance was involved only to a slight degree, the abnormalities consisting of a few widely spaced perivascular hemorrhages and thrombosed small blood vessels.



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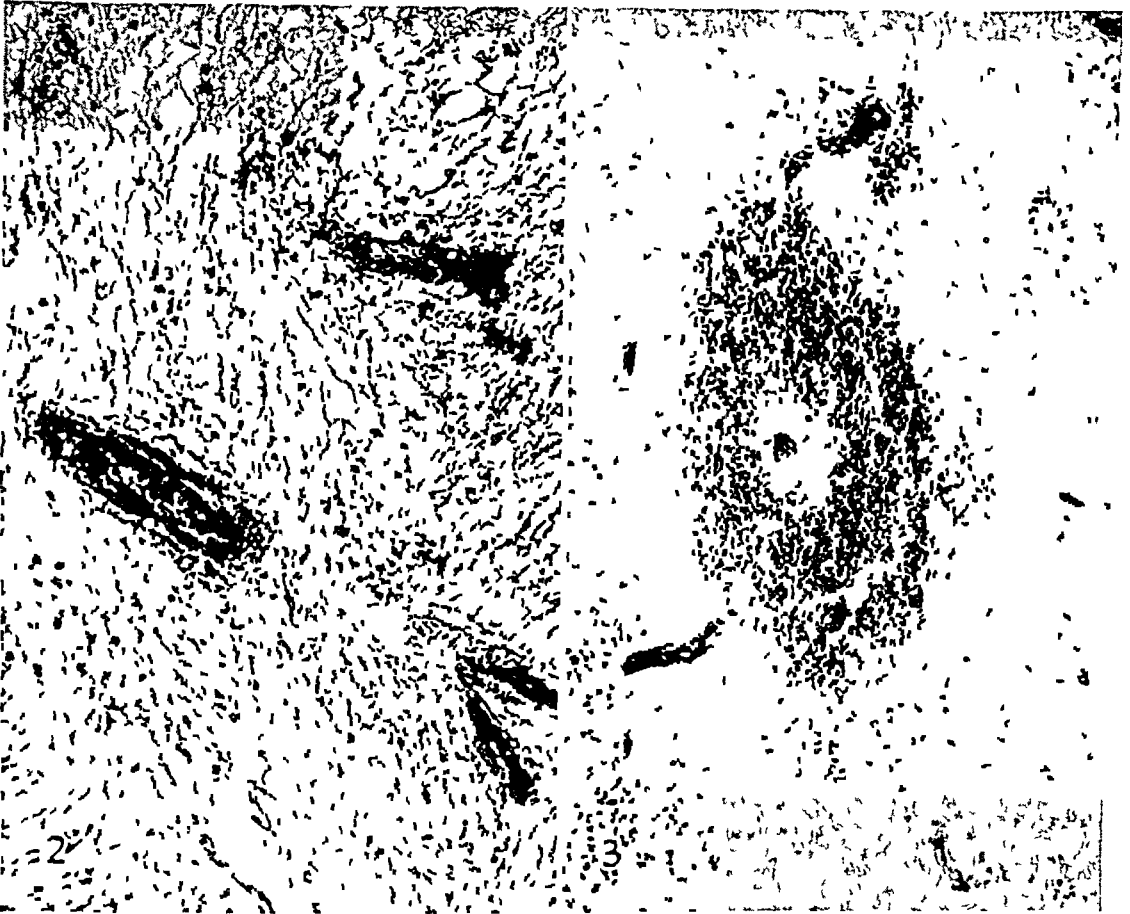


Fig 1—Bilateral hemorrhagic necrosis of the pons

Fig 2—Section from area of claustrum, showing arteriolar necrosis, perivascular hemorrhage and thrombosis of capillary blood vessels Mallory's phosphotungstic acid-hematoxylin, $\times 77$

Fig 3—Section of corpus callosum Note necrosis and thrombosis of small blood vessels and vacuolation associated with demyelination Mallory's phosphotungstic acid-hematoxylin, $\times 385$

Corpus Callosum and Adjacent Cortex The lesions were most advanced in the white substance and involved particularly the lateral portions of the corpus callosum. On each side there was an irregular area of necrosis within which all of the small blood vessels were occluded by thrombi. The necrotic tissue was fenestrated and lightly infiltrated by neutrophils and a few lymphocytes. In broad zones surrounding the necrotic areas and in the white substance of the central portion of the corpus callosum there were numerous areas of perivascular hemorrhage which varied greatly in size (fig. 3). The ependymal cells lining the ventricles and covering the choroid plexus showed no definite pathologic alteration. The small areas of gray cortex were essentially normal. The interstices of the arachnoidal membrane were infiltrated by well preserved erythrocytes.

Rostral Portion of the Pons In the dorsal one third there were no lesions except irregularly scattered small perivascular hemorrhages involving both the nuclear masses of the middle portion of the pons and the brachium conjunctivum. In the ventral two thirds these hemorrhages were both more numerous and larger and tended to surround two large areas of necrosis, one on each side of the midline. The latter areas were irregular, about equally extensive on the two sides but not exactly symmetric. Near the center of the hemorrhagic patches, small thrombosed blood vessels were surrounded by zones of necrosis. The walls of some of the larger vessels appeared to be necrotic, and these were infiltrated by neutrophils. Similar leukocytic infiltrates surrounded some of the occluded hyalinized arterioles and capillaries. Many thrombosed blood vessels lay in the seminecrotic tissue which surround the larger necrotic areas. Evidence of glial proliferation was slight.

Pons, Middle One Third, Including Root of Trigeminal Nerve The ventral three fourths of the pons was involved in a process similar to that described in the previous level. On one side an almost round area of necrosis extended from the ventral surface dorsally and crossed the median line. A second area, quite irregular in outline, was located on the opposite side and extended from the ventral surface dorsally to the junction of the middle and dorsal thirds. The small cavities formed in these areas were largely occupied by necrotic tissue and blood. Many small blood vessels showed necrosis and infiltrating neutrophilic leukocytes and were surrounded by broad irregular zones of hemorrhage. The degenerated tissue which formed the walls of the cavities was coarsely vacuolated. In the dorsal one fourth a few of the larger blood vessels were occluded by thrombi and surrounded by zones of necrosis of brain tissue with loss of substance. No gliosis was evident in this section.

Medulla, Upper Level Two small areas of perivascular hemorrhage were located just beneath the floor of the fourth ventricle, one on each side. The olivary nuclei were not seriously involved, but the white substance of the hilus contained a few venules which were partly collapsed and surrounded by narrow zones of hemorrhage and edema. A small area of anemic necrosis with light interstitial hemorrhage lay just ventral to one of the olivary bodies.

Spinal Cord It was essentially normal.

Hypophysis No lesions were recognized.

Chemical Analysis—The Gutzeit test revealed 0.6 mg. of arsenic per hundred grams of brain tissue.

Bacteriologic Examination—The spinal fluid gave no growth.

COMMENT

According to Kinnier Wilson,³ 50 per cent of deaths caused by arsenicals in all countries have been due to "hemorrhagic encephalitis" Ingraham⁴ reported 7 deaths due to arsenic, 1 from encephalopathy that was demonstrated at autopsy. However, in his review of 35 fatal arsenical reactions, 27, or 64 per cent, of the patients were said to have died from arsenical encephalopathy. Burton and associates⁵ reported 18 deaths due to arsenic, 3 of which resulted from cerebral complications. Woods⁶ reported 12 fatal poisonings, 6 of which had resulted in "hemorrhagic encephalitis". Stokes⁷ observed 1 arsenical reaction in 63,000 injections, Glaser and associates⁸ in a review found 1 death due to encephalopathy in every 5,398 patients treated and 1 in every 28,768 injections. It has been suggested that the cerebral lesions occur much more frequently than statistics indicate.⁹

Males are more often affected. Of the 17 females whose cases have been reported, 8 were pregnant. Pregnant women are more subject to the disastrous as well as to the beneficial effects of antisyphilitic therapy than are other persons. Cole's¹⁰ statistics indicate that patients with latent syphilis are more prone to reactions than those with early lesions, and a latent infection is the common finding in pregnant women.

Not all arsenical encephalopathy is found in syphilitic patients. Several reports have appeared with records of typical clinical and pathologic observations in cases in which the patients were treated with arsenicals for other diseases. Glaser and associates⁸ reported encephalopathy in a case of multiple sclerosis in which an organic arsenical was given. Burton and co-workers⁵ and Smith and Newbill¹¹ reported cases of Vincent's infection in which the patients were treated with arsenic, and Warach and associates¹² described a case in which a patient

3 Wilson, K S A. *Neurology*, London, Edward Arnold & Co, 1940

4 Ingraham, N R. *J A M A* **112** 1537, 1939

5 Burton, O L, Justyn, G W, and Anderson, L T. *U S Nav M Bull* **46** 139, 1946

6 Woods, E B. *J M A Georgia* **25** 23, 1946

7 Stokes, J H. *Modern Clinical Syphilology*, ed 2, Philadelphia, W B Saunders Company, 1934

8 Glaser, M A, Imerman, C P, and Imerman, S W. *Am J M Sc* **189** 64, 1935

9 Nelson, R B, McGibbon, C, and Glyn Hughes, F. *Brit M J* **1** 661, 1943

10 Cole, H N. *J A M A* **97** 897, 1931

11 Smith, D C, and Newbill, H P. *South M J* **32** 381, 1939

12 Warach, G S, Stone, L, and Kessler, M M. *Bull Meninger Clin* **1** 385, 1936

treated for acute cystitis died of "arsenical encephalopathy" following the administration of arsenic. Ecker and Kernohan¹³ described the pathologic observations in 12 cases in which the patients died with symptoms of diffuse cerebral damage. None of them had syphilis, and none was known to have received organic or inorganic arsenic, yet chemical assay of the brains revealed 0.1 to 0.83 mg of arsenic per hundred milligrams of tissue. (The concentrations were similar to those found in cases of arsenical encephalopathy.) They studied also 12 cases of hypertensive vascular disease with signs, symptoms and duration similar to those of the first group, and in none of the brains was an appreciable amount of arsenic found. It was concluded that these 12 cases of "subacute encephalomyelitis" represented instances of "chronic arsenical poisoning." The source of the arsenic was assumed to be food, water, etc. No theory as to why hypersensitivity developed in these particular persons was offered. A similar study with similar results was carried out by Osterberg and co-workers,¹⁴ who concluded that in cases in which the clinical picture is one of "hemorrhagic encephalitis" arsenic should be considered as the etiologic agent.

Signs and symptoms of the disease usually appear about forty-eight hours after the second or third injection of the first course of injections of an organic arsenical. Headache, confusion and irritability are the first and most common symptoms, followed by nausea and vomiting, progressive stupor, convulsions, hyperpyrexia and death. Signs of injury of the central nervous system are, in order of their frequency: changes in deep tendon reflexes, loss of corneal reflex, disturbance of speech, tremors, loss of light reflex, hemiplegia, nystagmus and facial palsy. Meningismus is rare. In patients who recover, bizarre neurologic findings and transient mental aberrations are seen.

The blood picture is usually within normal limits if infection has not intervened. The urine may show albumin (1 to 3 plus), but the degree of albuminuria is not correlated with the degree of damage of the central nervous system. Microscopic hematuria is often seen, as would be expected in cases of generalized increase of capillary permeability. Cerebrospinal fluid pressure is initially normal, but later, as cerebral edema occurs, the pressure tends to rise. The spinal fluid protein is elevated in cases of the most severe types.

13 Ecker, A. D., and Kernohan, J. W. *Arch Neurol & Psychiat* **45** 24, 1941

14 Osterberg, A. E., Brunsting, L. A., and Montgomery, H. *Proc Staff Meet, Mayo Clin* **10** 152, 1935

The mortality rate is high, approximately 75 per cent of recognized cases terminating in death, which occurs usually within five to seven days after the last injection of arsenic. However, several recoveries have been reported¹⁵

Pathologically, the majority of the patients whose cases have been reported have shown similar changes in varying degrees. Cormia¹⁶ stated, "The different syndromes are expressions of varying degrees of severity of the same essential process."

The lesions show a predilection for the white substance of the brain, with only mild congestion in the gray matter. The classic description by Kinnier Wilson³ leaves little to be added.

Hemorrhagic encephalitis is distinguished by abundant small perivascular effusions, mostly of the ring type, plugged capillaries with minute necrotic zones around them and sometimes incomplete partial occlusion in larger vessels, on occasions these thrombi are hyaline. Interstitial reactions otherwise (cellular infiltration of vessel coats rather than of perivascular spaces) are never pronounced and frequently lacking.

Subpial extravasation may be present in the optic thalami, cornu ammonis, tegmentum, pons and elsewhere. At times, however, nothing more than congestion and edema is discovered. Moderate demyelination around hemorrhagic zones is also seen occasionally.

The process probably begins with congestion and edema, followed by thrombosis, hemorrhage, demyelination, gliosis and ultimately necrosis of the vessel wall and surrounding brain substance, with occasional progression to cyst formation. The pericapillary hemorrhages or "ring hemorrhages" often coalesce to form large bilaterally symmetric areas of hemorrhage. Certain areas are more susceptible than others, i.e., the corpus callosum, the caudate and lenticular nuclei, the pons and the medulla. However, hemorrhage has been reported to involve almost all of the brain substance. The bilateral symmetry of these lesions is a striking feature which has been commented on by many authors.

Almost identical gross and microscopic changes have been observed in many other conditions, including poisoning due to carbon monoxide and phosgene gas, tuberculosis meningitis, meningitis due to other bacteria, influenza, pneumococcal pneumonia, malaria, typhus, pernicious anemia, secondary anemia, erythema multiforme, psoriasis, scurvy, cere-

15 Thomas, E. W., Wexler, G., and Dattner, B. *Am J Syph, Gonorr & Ven Dis* **26** 529, 1942. Lees, D. *Practical Methods in the Diagnosis and Treatment of Venereal Disease*, ed 3, London, Butterworth & Co., 1937. Netherton, E. W. *M Clin North America* **17** 1005, 1934. Dickens, P. F. *U S Nav M Bull* **26** 192, 1928. Parnell, R. J. G., and Dudley, S. F. *Lancet* **1** 190, 1920. Gjessing, H. C. *Acta dermat-venereol* **8** 268, 1927.

16 Cormia, F. E. *Canad M A J* **35** 610, 1936.

bral thrombosis, cerebral trauma, and meningovascular syphilis, and they may be secondary to cerebral circulatory disturbances caused by hemorrhage, thrombosis or tumor

Pathogenesis—There is such an array of findings associated with arsenical encephalopathy that it is not surprising that no one theory yet proposed has proved entirely satisfactory. There have been many suggestions as to the genesis of this process, and one of the first and one which received much support in the early days was proposed by Ehrlich¹⁷. He was of the opinion that the damage of the central nervous system was not due to the direct effect of the drug, and he insisted that yellow atrophy of the liver alone should be considered as a direct effect of the drug intoxication. He maintained that the cerebral injury is an expression of a Herxheimer reaction. This theory, however, has since been disproved, since numerous cases of arsenical encephalopathy have been observed in persons who did not have syphilis¹⁸.

Pathologic evidence of arsenical encephalopathy occurring in the presence of acute encephalitis and parenchymatous cerebral syphilis has been described. However, the question of death due to a Herxheimer reaction must be raised⁴.

Brown and Pearce¹⁹ found that in animals given toxic doses of arsenic marked congestion, hemorrhage and necrosis of the adrenal glands developed. On the basis of the results of this study it has been suggested²⁰ that the vascular damage is the result of an insufficiency of epinephrine, the substance assumed to be necessary to counteract the vasodilating effect of arsphenamine. Epinephrine has been widely used in the treatment of this condition, almost always without avail.

Since symptoms occur most frequently after the second injection, it seems possible that the mechanism of the cerebral injury may be allergic in nature. Halcrow²¹ suggested that disintegration products of arsenic preparations have a toxic action on the capillaries. Thrombopenic purpura is a well known result of organic arsenical toxicity, and it is possible that the encephalopathy is due to disintegration of arsenobenzine compounds in the blood with resulting thrombopenia and capillary damage.

Friedman and Newman²² reported 2 cases of arsenical encephalopathy in which the patients were infants, 6 and 14 months of age. They offered the theory that there is a delay of metabolism of the arsenic due

17 Cited by Scheinker, I. M. *Arch. Path.* **37** 91, 1944.

18 Pritzi, O. *Zentralbl. f. Gynak.* **52** 2930, 1928. Glaser and others⁸. Smith and Newbill¹¹. Warach and others¹².

19 Brown, W. H., and Pearce, L. *J. Exper. Med.* **22** 535, 1915.

20 Vail, A. D. *J. Missouri M. A.* **38** 110, 1941. Cormia¹⁶.

21 Halcrow, J. P. A. *Brit. M. J.* **1** 663, 1943.

22 Friedman, E. D., and Newman, E. *J. Nerv. & Ment. Dis.* **94** 606, 1941.

to hepatic insufficiency and accumulation in the body of toxic products of the drug because of renal dysfunction. Thus hypersensitivity to arsenic or its metabolites develops, and the patient dies an anaphylactoid death when another injection is given.

Others¹⁷ have expressed the belief that the vascular lesions occur as a result of an acute physical or chemical irritation, and after vasoconstriction of short duration there soon occurs a paralytic dilatation of the affected vessel, usually associated with stasis. If the circulation is impaired because of this process, a local accumulation of carbon dioxide occurs and interferes with the oxygen supply to the capillaries. If these alterations become severe, degeneration of the vessel wall occurs, with increased permeability to serous fluid and red blood cells. Extravasation occurs and results in further stasis due to pressure and tissue starvation. Thus a vicious cycle is set up which eventually may result in degeneration and necrosis of the surrounding tissue.

Out of the confusion which exists concerning the genesis of arsenical encephalopathy, we may conclude that the mechanism is vascular in nature and that the pathologic changes, in most cases, are out of proportion to the apparent functional damage of the central nervous system.

SUMMARY

A patient was treated for early syphilis with penicillin, followed by neoarsphenamine, which was administered in two intravenous injections. Death occurred eight days after the last injection. Treatment with 2,3-dimercaptopropanol (BAL) was begun on the fifth day after the onset of cerebral symptoms (seven days after the last injection of neoarsphenamine) without apparent effect.

The cerebral lesions consisted of thrombosis of arterioles and capillary blood vessels, necrosis of arterioles, perivascular hemorrhages and hemorrhagic necrosis of brain substance. There was marked symmetry of the lesions, and the white matter was principally affected, especially the pons, the corpus callosum, the claustrum and the thalamus. An unusually high level of arsenic was found on chemical analysis of the brain.

The pathogenesis and the gross and microscopic lesions are discussed, and the available literature since 1933 is reviewed.

EFFECT OF EXPERIMENTAL THIAMINE DEFICIENCY ON THE NERVOUS SYSTEM OF THE RHESUS MONKEY

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DURING the course of studies of rhesus monkeys which had been subjected to one or more episodes of thiamine deficiency we observed, repeatedly, widespread degenerative lesions of gray matter, differing somewhat in distribution, extent and histopathologic nature from the Wernicke type of lesion. These observations have been reported in abstracts¹. Because in distribution these lesions resembled those encountered in certain diseases of the human central nervous system and varied from what is usually reported in thiamine deficiency, and because there was an absence of peripheral neuropathy in these animals, the findings are reported in detail and are compared with those previously reported observed in man and animals.

The diet, the laboratory findings and the response of the animals to deficiency episodes and thiamine supplements have been described separately,² as have the characteristic lesions of the heart³.

TECHNIC OF EXAMINATION

The formaldehyde-fixed brains of 7 monkeys were sliced coronally, and sections were taken at about 3 mm intervals throughout the basal ganglions and brain stem, as well as representative areas of cortex, and embedded for histologic study. The spinal cord was sectioned at segmental intervals and all sections examined except in the case of the first 3 monkeys, from which only random sections were taken. Sections of cord and of the sciatic nerve and some of its larger branches were fixed in Muller's fluid for osmic acid stains. The usual stains were employed with Laidlaw's connective tissue stain as a satisfactory substitute for the benzidine stain for capillaries.

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1 Rinehart, J. F., Friedman, M., and Greenberg, L. D. *Tr. Am. Neurol. A.* **72** 174, 1946. Rinehart, J. F., Greenberg, L. D., and Friedman, M. *Am. J. Path.* **23** 879, 1943.

2 Rinehart, J. F., Greenberg, L. D., and Ginzton, L. L. *Blood* **3** 1453, 1948.

3 Rinehart, J. F., and Greenberg, L. D. *Arch. Path.* **48** 89, 1949.

PATHOLOGIC OBSERVATIONS

Gross Findings—There were areas of softening in the corpus striatum (chiefly the putamen) in 4 monkeys. In 3 animals these confluent small rounded areas of 3 to 8 mm size had sharply defined borders, with the softened part tending to shrink away from the normal tissue. In the fourth the borders were poorly defined, and the softened areas

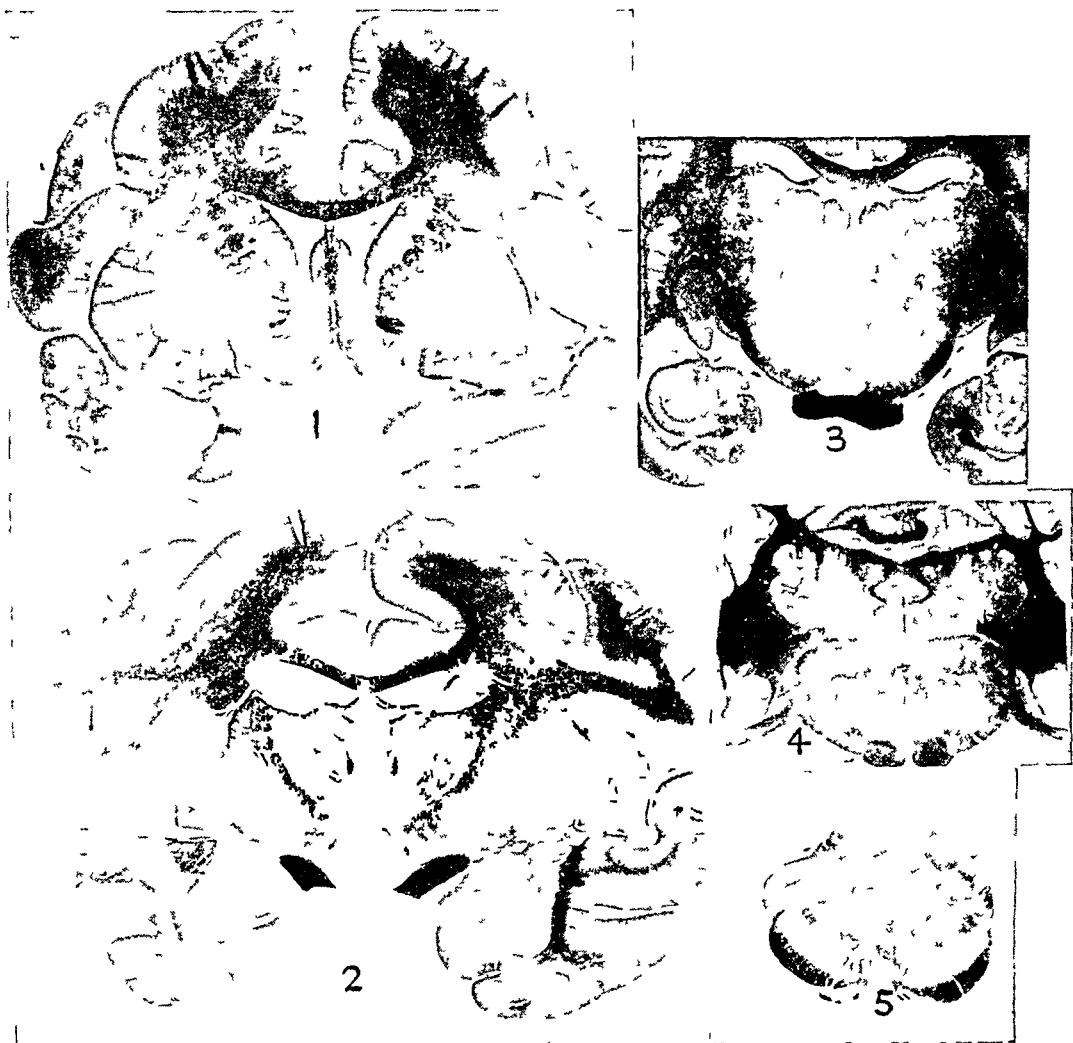


Fig 1—Symmetric degeneration of putamen, Weil stain, $\times 15$

Fig 2—Symmetric degenerations of lenticular nucleus, Weil stain, $\times 17$

Fig 3—Symmetric degenerations in substantia nigra and small lesions in median portion of thalamus, Weil stain, $\times 15$

Fig 4—Symmetric degeneration of nuclei of eighth cranial nerves, Weil stain, $\times 2$

Fig 5—Symmetric degenerations of nuclei of third cranial nerve, Weil stain, $\times 2$

had a very pale yellowish coloration and one or two concentric narrow pale rings. In 1 animal there were a few small areas of softening and pallor in the cortex of the left frontal lobe near the central fissure.

In the spinal cords, tiny reddish brown spots were seen occasionally in the gray matter

Histologic Observations—1 The earliest changes appeared to be localized edema of tissue, with fluid separating glial and nerve fibers, and fragmentation and disintegration of the myelin sheaths in this area. A few microglial cells were seen within such areas, and at the borders of the areas there were sparsely formed rings of these cells in various stages of transformation from the quiescent to the phagocytic form. The nerve cells were usually normal at this stage, which is designated as "early" in table 1.

2 A severe lesion of apparently rapid development was seen sometimes in the putamen and the substantia nigra and consisted of slight edema, demyelination, loss of staining qualities in all tissue elements and necrosis and liquefaction of most nerve cells. The nerve cells still present in these areas had acidophilic, granular cytoplasm and pale, weakly acidophilic nuclei. A scanty margin of microglial cells and a few astrocytes were seen at the borders of such areas. This change was not unlike an anemic infarct and is designated as "early severe" in table 1.

3 With further progression of either type of change there were found disintegration of axis-cylinders, degeneration of glia and progressive accumulation of many microglial cells and a few astrocytes. Usually several of the nerve cells (but sometimes surprisingly few) had undergone acute swelling or severe cell disease with disintegration (figs 6 and 9). This stage is designated as "moderately advanced, focal" in table 1. None of the animals was maintained sufficiently long after reaching the deficient stage to carry the changes to the point where scars or extensive gliosis occurred.

4 Around, and particularly between, some of the older lesions in the corpus striatum there was a diffuse, poorly demarcated area of changes consisting of degeneration of some nerve cells and some fibers and marked proliferation of astrocytes, many of which were beginning to assume the gemastete appearance.

Vascular changes conforming somewhat to those seen in the Wernicke type of lesion, but without petechiae, and consisting of dilated, tortuous capillaries with some endothelial proliferation and sometimes actual new vessel formation were occasionally seen (fig 7). They occurred only in the oldest, severest lesions in those animals which were on the most prolonged deficiency regimen. They were noted chiefly in the putamen and never in the corpora mamillaria or the thalamus, and they appeared to be an attempt at repair of injured tissue rather than a primary lesion. The Laidlaw connective tissue

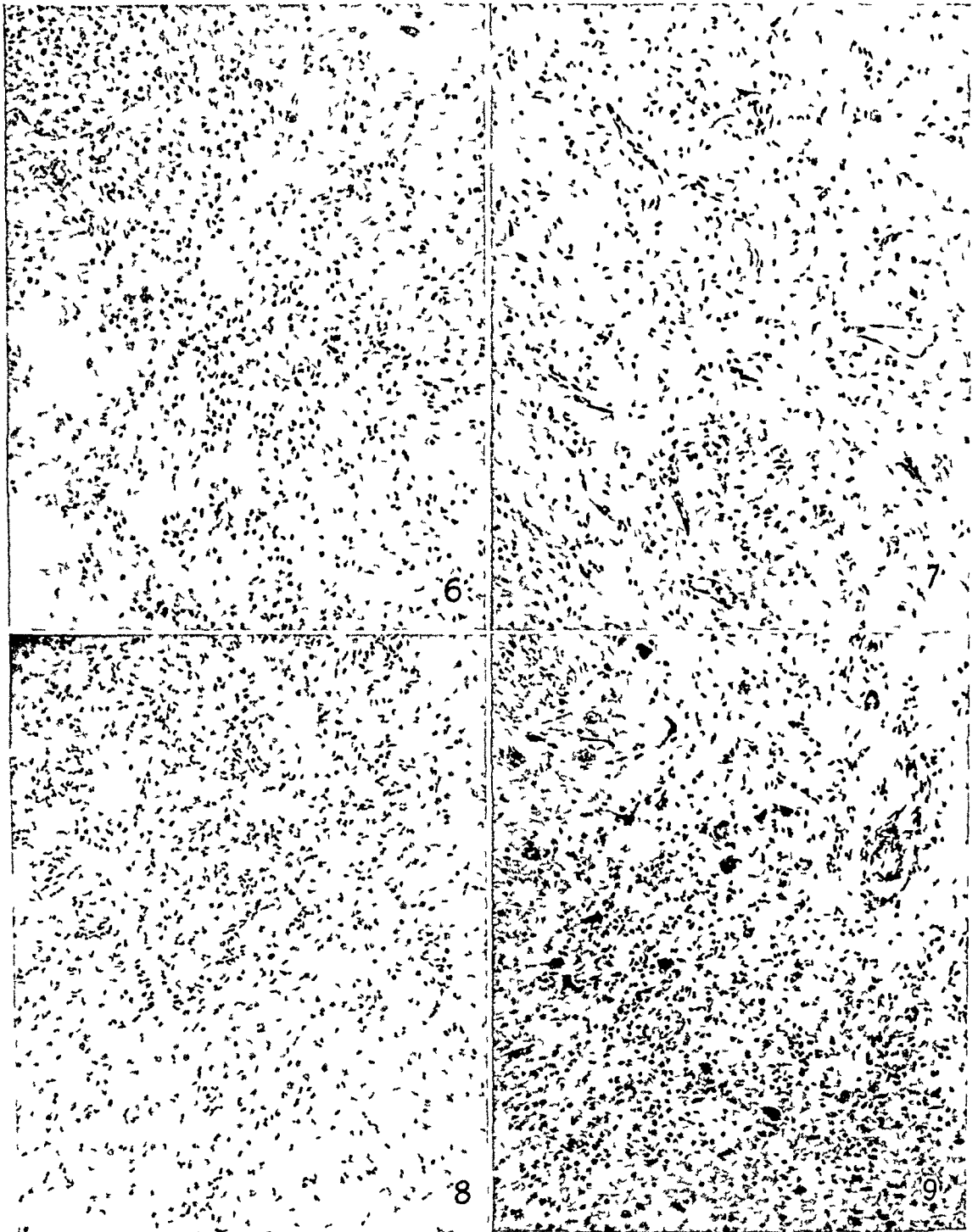


Fig 6—Inflammatory and scavenger cells at edge of region showing advanced stage of degeneration in putamen, hemalum-eosin stain, $\times 80$

Fig 7—Vascular dilatation and proliferation and inflammatory and scavenger cells near edge of older lesion in putamen, hemalum-eosin stain, $\times 80$

Fig 8—Inflammatory cells in degenerating portion of inferior colliculus of corpora quadrigemina, hemalum-eosin stain, $\times 80$

Fig 9—Inflammatory cells and degenerating neurons in nucleus of third cranial nerve, hemalum-eosin stain, $\times 125$

stain and even the hematoxylin-eosin stain proved generally satisfactory for studying the vascular changes

In the spinal cord there were some petechiae, usually in the gray commissure, in about half of the animals

In the peripheral nerves (and in random distribution in the spinal cord) there were a few scattered black particles in Marchi-stained sections, sometimes in myelin sheaths and sometimes outside. Axis-cylinders were normal, myelin sheaths appeared normal in hematoxylin-eosin, fat, myelin and other stains, no inflammatory cells or

TABLE 1—*Lesions in 7 Monkeys*

	Animals		Type of Lesions	Degeneration of Nerve Cells
	Num ber	Per centage		
Caudate nucleus	4	57	Early severe, advanced focal and advanced diffuse	Severe
Putamen	5	71	Same as above	Severe
Globus pallidus	2	28	Early severe	Mild
Thalamus, median nucleus	2	28	Early severe, advanced focal	Moderate
Substantia nigra	1	14	Early severe	Moderately severe
Nucleus centralis superior	2	28	Advanced focal	Mild
Mamillary bodies	1	14	Moderately early focal	Mild
Inferior colliculi of tectum	4	57	Early and advanced focal	Mild to moderate
Nucleus fasciculus solitarius	2	28	Early	None
Cranial nerve III (oculomotor) nucleus	3	43	Advanced focal	Mild to moderate
Cranial nerve VI (abducens) nucleus	3	43	Advanced focal	Mild
Cranial nerve VIII (auditory) nucleus	2	28	Advanced focal	Moderate
Cranial nerve X (vagus) nucleus, dorsal	3	43	2 early, 1 Wernicke (no 353)	Slight
Nucleus parvocellularis annuli aqueductus	1	14	Early	None
Cerebellar vermis	4	57	Moderately advanced focal	Mild
Cerebral cortex	1	14	Early severe	Moderately severe
Spinal cord, gray substance	3 or more		Rare petechiae only	None

phagocytes were seen, and it was concluded that the black particles in the Marchi stain, unlike "Marchi balls" in appearance and distribution, were artefacts. The peripheral nerves were therefore considered normal. Myoneural junctions were studied in 1 case and were found to be histologically normal.

Distribution of the Lesions These lesions were entirely restricted to the gray matter of the central nervous system, chiefly to the basal ganglions and the brain stem. They were almost always bilateral and usually so nearly symmetric and bilaterally equal as to be "mirror images" of each other. The largest, most consistently occurring and most striking lesions were in the corpus striatum (figs 1 and 2),

where degeneration of nerve cells was also generally most extensive. The cerebral cortex was involved in only 1 animal, and small foci were found in the vermis of the cerebellum in 4. Table 1 summarizes the distribution of the lesions.

CORRELATION OF LESIONS WITH BEHAVIOR OF ANIMALS

The common result of deficiency in all animals was profound weakness, some had ataxia, and a few had focal signs. The correlation of the lesions with definite signs in some animals was as follows:

<i>Lesions in</i>	<i>Signs</i>
Corpus striatum, cranial nerve VI (abducens) nucleus, cerebellar cortex	Ataxia
Inferior colliculus, nucleus fasciculus solitarius	Ataxia
Corpus striatum, cranial nerve X (vagus) nucleus, cerebellar cortex, cranial nerve VI nucleus	Localized tonic convulsion, tends to walk in circles
Corpus striatum, globus pallidus, thalamus, inferior colliculi, cranial nerve VIII (auditory) nucleus, subthalamic body	Ataxia, hypersensitivity to auditory stimuli
Corpus striatum, globus pallidus, thalamus, mammillary bodies, cranial nerve III (oculomotor), VI and X nuclei, cerebellar cortex	Ptosis
Corpus striatum, inferior colliculi, cranial nerve III nucleus, cerebellar cortex	Ptosis, ataxia

Although specific clinical findings were meager, they did agree fairly well with anatomic findings, for example, the ptosis in 2 animals with severe lesions of the oculomotor nuclei, and the hypersensitivity to sound of 1 animal with a lesion in the eighth cranial nerve nucleus. The small size of cerebellar cortical lesions seemed inadequate to explain the ataxia unless degeneration in one area of cortex may signify a widespread predegenerative paralysis of other cerebellar cortical cells.

COMMENT

Nature of Degenerative Process—The lesions resemble, in most instances, anemic infarction or degeneration such as ordinarily follows subtotal occlusion or temporary spasm of an artery. The ischemic changes of the nerve cells, the degeneration of nerve fibers, the circumscribed nature of the lesion and the cellular reaction at the edges of the larger lesions all point toward this resemblance. However, neither thrombus nor other vascular occlusive process could be demonstrated histologically. Vasospasm could easily produce the observed changes, but use of this hypothesis would necessitate further explanations for the occurrence of bilateral, exactly symmetric lesions, the avoidance of white matter (which is commonly involved in such infarcts in man) and the relative resistance of nerve cells. A more acceptable,

although as yet unsupported, explanation might be based on a purely chemical change, such as accumulation of an excess of pyruvic acid or other metabolites

Explanation of Localization—The restriction of the observed lesions chiefly to the axial gray matter of the central nervous system has, as yet, no obvious explanation. Bender and Schilder,⁴ in discussing encephalitis hemorrhagica as observed in alcoholism, stated their belief that the lesions were associated with the flow of cerebrospinal fluid and were most severe where there was stagnation of fluid. Subsequent observations, and particularly the finding of large areas of devastation in the putamen in most of our animals, refute this concept. The finding of lesions of the striatum and striopallidum and most particularly of the putamen itself emphasizes again the general susceptibility of that region to many diseases of known and unknown cause (Wilson's disease, Parkinson's disease, Huntington's chorea, kernicterous, manganese, carbon monoxide and carbon disulfide poisoning), as yet unexplained. Hiller's⁵ theory of electivity of diseases may be of some help. In his opinion susceptibility of a tissue to disease is determined by the paucity of the blood supply or by a high rate of metabolism. He mentioned the relatively poor blood supply to the corpus striatum (due partly to the scanty vascular tree and partly to acute angulation of the vessels, which impedes the flow). We are tempted to carry his concept one step further and consider the susceptibility of tissue to be related to the ratio of metabolism to vascularity. This would explain in our present experiments the selectivity for the gray matter, which has greater vascularity but much greater metabolic activity than the white matter, and the relative sparing of the cerebral cortex, which may have a considerably better vascularity than the subcortical gray matter.

Relation to Wernicke's Encephalopathy—The lesions of the central nervous system described in the foregoing pages correspond in a general way to the polioencephalitis hemorrhagica of Wernicke in human subjects and its equivalent in many experimental animals restricted to thiamine-deficient diets. Points of similarity are the symmetry of the lesions, their restriction to gray matter and their occurrence in periaqueductal and periventricular regions. Points of dissimilarity are the relatively extensive and severe lesions in the corpus striatum, the relative rarity and small size of the thalamic lesions and the relative absence of the vascular changes which have been considered so important a part of the process in polioencephalitis hemorrhagica superior.

4 Bender, L., and Schilder, P. Arch Neurol & Psychiat 29 990, 1933

5 Hiller, F. Arch Neurol & Psychiat 20 145, 1928

A comparison of the distribution of lesions of this type in various animals and in man is presented in table 2

In comparing the histologic nature of the lesions in our animals with those of Wernicke's polioencephalitis hemorrhagica, we find first the need for clarification of one point namely, the significance, if any,

TABLE 2—*Comparison of the Distributions of the Lesions in Man and Various Other Animals*

Location	Man *	Monkey	Fox †	Rat ‡	Pigeon §	Pigeon
Cerebral cortex	++	+	+			
Septum pellucidum	++					
Corpus callosum	+					
Cerebellar cortex	+	+++	+++		\	
Basal ganglions						
Caudate and putamen	+	++++			\	
Globus pallidus		++	++			
Thalamus	++++	++	++			x
Habenular nucleus	+		++			
Substantia nigra	+	+			+	
Hypothalamus	++++					x
Dorsomedial nucleus	+++					.
Ventromedial nucleus	++++					
Supraoptic nucleus	+					
Corpora mamillaria	++++	++				x
Midbrain gray matter	+++					
Superior colliculi	++		+			
Inferior colliculi	++		+++			
Nucleus centralis superior		++				
Cranial nerve III (oculomotor) nucleus	++	+++	+++		\	\
Cranial nerve IV (trochlear) nucleus	+		+		\	\
Pons and medulla						
Cranial nerve VI (abducens) nucleus	++	+++		++		x
Cranial nerve VIII (auditory) nucleus	+++	++	+++	++++	++	x
Cranial nerve X (vagus) nucleus	+++	+++	+++			\
Bechterew's nucleus				+++		
Nucleus fasciculus solitarius		++		++++		
Restiform body			+++			
Inferior olivary nucleus	++					
Cuneate nucleus and nucleus gracilis			+++			
Locus caeruleus	+					
Nucleus pontis	+					
Deiters' nucleus				++++		

+ to ++++ indicate rare to very common \ indicates that lesions were "present," frequency not stated

* The data are drawn from Bender and Schilder⁴ Hiller⁵ Campbell and Biggart⁷ Jolliffe and others⁹ Meyer, A. J. *Neurol & Psychiat* 7 66, 1944 Riggs, H. F., and Boles R. S. *Quart J Stud on Alcohol* 5 361, 1944 de Wardener, H. E., and Lennox, B. *Lancet* 1 * 11, 1947

† The data are from Evans, C. A., Carlson, W. E., and Green, R. G. *Am J Path* 18 79, 1942

‡ The data are from Prickett¹⁷

§ The data are from Swank and Prados¹⁰

|| The data are from Alexander⁶

of the capillary changes in the latter Throughout the earlier literature on human and experimental Wernicke lesions, much emphasis was placed on the capillary dilatations, varicosities, and tortuosity, on

the endothelial proliferation and on the petechial hemorrhages Alexander⁶ decided that this was the primary lesion, with parenchymal degeneration a relatively less important result, and even adopted the benzidine stain for capillaries as the standard technic for studying this disease. However, the parenchymatous necrosis in human cases has received some emphasis by Campbell and Biggart⁷ and Zimmerman⁸. Jolliffe and associates⁹ stated that hemorrhages are not always present. Swank and Prados,¹⁰ using the same species as Alexander (pigeon), decided that the parenchymal necrosis was primary and the vascular lesions secondary. Folts,¹¹ in discussing the methods and conclusions of Alexander, stated that Alexander "has postulated without any justification whatsoever, that thiamine possesses 'angio-degenerative properties'". Folts, in fact, doubted that Wernicke's encephalopathy is a result of thiamine deficiency alone and considers questionable the published data on this disease in man, the pigeon and the fox. Dunn¹² found hemorrhages in 60 per cent of thiamine-deficient mice, usually in the medulla, but no evidence of any vascular proliferation. Hemorrhages seemed to have a predilection for those areas which are normally quite vascular and have thin-walled blood vessels.

In our own animals the vascular changes were not found at all in the earlier and milder lesions and only in moderate degree in the more severe lesions of animals with moderately prolonged deficiency diet. Only in the animal with the greatest number of acute deficiency episodes (illustrated in figure 7) and the animal with a continuous prolonged deficiency state was the vascular change fairly prominent, consisting of dilatation, endothelial proliferation and an apparent true increase in the number of vessels and capillary budding in the corpus striatum. The vascular activity seemed to be a response to, rather than the cause of, the destruction of nerve tissue, being the principal attempt at repair observed.

Human vitamin deficiencies are usually multiple, and it is probable that most of the earlier experimental work on thiamine also involved other deficiencies. Therefore, it seems likely, by implication

6 Alexander, L. *Am J Path* **16** 61, 1940

7 Campbell, A. C. P., and Biggart, J. H. *J Path & Bact* **48** 245, 1940

8 Zimmerman, H. M. *A Research Nerv & Ment Dis, Proc* (1941) **22** 51, 1943

9 Jolliffe, N., Wortis, H. and Fein, H. D. *Arch Neurol & Psychiat* **45** 569, 1941

10 Swank, R. L., and Prados, M. *Arch Neurol & Psychiat* **47** 97, 1942

11 Folts, R. H., Jr. *The Pathology of Nutritional Disease*, Springfield, Ill., Charles C Thomas, Publisher, 1948

12 Dunn, T. B., Morris, H. P., and Dubnik, C. S. *J Nat Cancer Inst* **8** 139, 1947

that the vascular changes of human polioencephalitis hemorrhagica superior are either the result of other deficiencies or only a secondary occurrence in thiamine deficiency, and that the characteristic result of thiamine deficiency in man, primates and probably other animals is primary parenchymatous degeneration of subcortical gray matter

Peripheral Neuropathy—The absence of evidence of peripheral neuropathy is another item which deserves special mention and clarification. Contradictory reports on the relation of thiamine to neuritis have continued to appear during the past decade and are due in part to greater difficulties in dietary control in the earlier work, to effects of starvation in some cases and most of all to the diversity of criteria set up by various workers for the diagnosis of neuritis. In regard to criteria, we find at one end of the scale such generous interpretations as that of Lowry, Sebrell, Daft and Ashburn,¹³ who diagnosed polyneuropathy when a rat became spastic and had an ataxic tonic convulsive seizure when dropped on its back from a 3 to 5 inch (7.5 to 12.5 cm) height, or that of Prickett, Salmon and Schrader,¹⁴ who diagnosed neuropathy on the basis of an increase in the amount of isotropic material in myelin sheaths, even though the Marchi and other stains were negative. At the other end of the scale, Swank and Prados¹⁵ required alterations of axis-cylinders, as well as easily demonstrable degeneration of myelin sheaths, for the diagnosis of neuropathy.

In regard to starvation, always a potential factor in thiamine deficiency, degeneration of myelin may result from it alone, according to Williams and Spies,¹⁶ Swank¹⁶ and Swank and Prados.¹⁵ Prickett, Salmon and Schrader,¹⁴ using the polarized light technic, actually found slightly more alterations of myelin in rats fed adequate amounts of thiamine and limited as to food intake than in the acutely thiamine-deficient rats, although this did not hold true in chronic deficiency. Absence of any significant peripheral neuropathy in thiamine-deficient animals has been reported by Prickett¹⁷ in rats, by Wechsler, Jervis and Potts¹⁸ in monkeys, by Engle and Phillips¹⁹ in chicks, by

13 Lowry, J. V., Sebrell, W. H., Daft, F. S., and Ashburn, L. L. *J. Nutrition* **24** 73, 1942.

14 Prickett, C. O., Salmon, W. D., and Schrader, G. A. *Am. J. Pathol.* **15** 251, 1939.

15 Williams, R. R., and Spies, T. D. *Vitamin B₁ and Its Use in Medicine*, New York, The Macmillan Company, 1938, chap. 4, p. 57.

16 Swank, R. L. *J. Exper. Med.* **71** 683, 1940.

17 Prickett, C. O. *Am. J. Physiol.* **107** 459, 1934.

18 Wechsler, I. S., Jervis, G. A., and Potts, H. D. *Bull. Neurol. Inst. New York* **5** 45, 1936.

19 Engle, R. W., and Phillips, P. H. *J. Nutrition* **16** 585, 1938.

Wintrobe, Follis, Humphreys, Stein and Lauritsen²⁰ in pigs and by Dunn and associates¹² in mice. The suggestion that human peripheral neuritis is due to a multiple deficiency is made by participants in the discussion following the paper by Kolb and co-workers²¹ and by Strauss²². Zimmerman²³ in 1939 referred to thiamine as the "proven" antineuritic vitamin, but in 1943⁸ he stated that it is probably, but not necessarily, the antineuritic vitamin in man. Meiklejohn²⁴ summarized the clinical and experimental reports up to 1940 and concluded that thiamine is not the "antineuritic vitamin". Follis¹¹ concluded that thiamine is not antineuritic in lower mammals but may possibly be so in birds. In summary, then, no histologic evidence of peripheral neuritis was found in these 7 monkeys, and this finding is in agreement with the results of many other recent investigations of experimental thiamine deficiency. Clinical evidence of sensory neuritis was not detected in our animals. However, the therapeutic value of thiamine in peripheral neuritis in man cannot be ignored, nor can we overlook the indispensable role of thiamine in normal nerve function, as recently summarized by Von Muralt²⁵. It is possible that there is a physiologic defect that is not reflected in structural changes.

SUMMARY

Feeding of 7 rhesus monkeys a thiamine-deficient diet with widely spaced small thiamine supplements resulted in bilateral symmetric areas of degeneration, observed most commonly in the corpus striatum and seen also in the globus pallidus, substantia nigra, mamillary bodies, corpora quadrigemina, cerebellar cortex and the nuclei of the third, sixth, eighth and tenth cranial nerves. Capillary and endothelial changes were a relatively late finding and were interpreted as an attempt at repair of the injury. Associated with these lesions were profound weakness, ataxia and occasional focal signs of cranial nerve weakness or hyperirritability. No clinical or histologic evidence of peripheral neuropathy or of degeneration of fibers of the spinal cord was observed.

20 Wintrobe, M. M., Follis, R. H., Jr., Humphreys, S., Stein, H., and Lauritsen, M. J. *Nutrition* **28** 283, 1944.

21 Kolb, L. C., Wintrobe, M. M., Mushatt, C., Miller, J. L., Jr., Lisco, H., and Stein, H. J. *Tr. Am. Neurol. A.* **67** 189, 1941.

22 Strauss, M. B. *A. Research Nerv. & Ment. Dis., Proc.* (1941) **22** 141, 1943.

23 Zimmerman, H. M. *Yale J. Biol. & Med.* **12** 123, 1939.

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PATHOLOGIC CHANGES AFTER PARTIAL HEPATECTOMY

With Special Reference to Hepatic Necrosis in Protein-Depleted Rats

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THIS report is based on observations made during the course of experiments in which partial hepatectomy was performed on 230 rats. A detailed protocol was kept at autopsy of each rat that died or was killed, and histologic sections were prepared of the liver at operation and of the liver and certain other organs at the time at which an animal was killed. The analytic and metabolic data for which the experiments were designed have been reported elsewhere¹. The most important pathologic finding is considered to have been a severe focal necrosis of the liver which appeared in some of the animals that had been partially depleted of protein before the operation.

METHOD

The animals used were all male Wistar rats weighing approximately 250 Gm. Details of diets and methods have been described elsewhere¹. The operation of Higgins and Anderson,² which in our hands removed 69.4 ± 1.34 per cent of the liver, was employed in all but 12 animals. Ether anesthesia was used. Regeneration was studied after postoperative intervals of one to fourteen days. Tissue for histologic examination was fixed in 4 per cent formaldehyde solution and stained with hematoxylin and eosin and with sudan III for fat.

PATHOLOGIC CHANGES IN THE LIVER

In table 1 the animals are divided into six series according to their management. Fifty-six animals (series 1) came to operation in good nutrition, having been reared on a stock diet until one week before operation, when a synthetic diet containing 18 per cent or more of casein was substituted. No complications beyond occasional infection of a wound was observed among the 52 animals which survived operation. There were no postoperative deaths, and no gross

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1 (a) Gurd, F N, Vars, H M, and Ravdin, I S. *Am J Physiol* **152** 11, 1948. (b) Vars, H M, and Gurd, F N. *ibid* **151** 391 and (c) 399, 1947.

2 Higgins, G M, and Anderson, R M. *Arch Path* **12** 186, 1931.

or microscopic abnormalities were noted in the livers. Mitotic activity paralleled that reported by Brues, Drury and Brues³. Mitoses appeared between eighteen and twenty-four hours after operation, were still numerous at forty-eight hours, much fewer at four days and difficult to find thereafter. This sequence applied to both well nourished and protein-depleted animals. It was our impression that twenty-four and forty-eight hours after operation the mitoses were actually more numerous in the livers of protein-depleted animals than in those of the well nourished series.

Chemical analysis of the liver tissue removed at operation from the protein-depleted animals (series 2, 3, 4 and 6), which had been consuming a high carbohydrate diet and were not fasted, showed total lipid to be 6 to 8 per cent and glycogen 6 to 9 per cent^{1a}. In gross appearance the liver tissue was paler than that of the protein-fed animals. Histologically, the liver cells were moderately

TABLE 1—*Causes of Deaths Following Partial Hepatectomy in Various Dietary Groups of Rats*

Series	Pre operative Diet	Operative Treatment	Rats Operated on	Number That Survived Operation	Number That Died Post operatively	Causes of Postoperative Deaths		
						Hepatic Causes		Other Causes
						Massive Necrosis	Focal Degeneration	
1	Adequate protein	Hepatectomy, 69.4%	56	52	0	0	0	0
2	Nonprotein 14 days	Hepatectomy, 69.4%	144	141	15	6	4	Pneumonia—2 Unknown—3
3	Nonprotein 28 days	Hepatectomy, 69.4%	6	6	1	0	0	Pneumonia—1
4	Nonprotein 14 days	Hepatectomy, 69.4%, and NaR*	6	6	2	0	2	0
5	Stock	Hepatectomy, 77%	6	5	0	0	0	0
6	Nonprotein 14 days	Hepatectomy, 77%	12	11	4	0	2	Pneumonia—1 Unknown—1
Total			230	221	22	6	8	6

* Sodium ricinoleate, 0.5 cc of a 10 per cent solution per hundred grams of body weight, was injected subcutaneously.

large with apparently normal nuclei. The cytoplasm was extensively vacuolated, with no deep-staining basophilic particulate granules. Figure 1 illustrates such a specimen of tissue. The photomicrograph is identical in appearance with the photomicrographs of liver from hypoproteinemic dogs presented by Elman and Heifetz⁴. More recently, in an important contribution, Kosterlitz⁵ illustrated histologic sections from the livers of rats fed protein-deficient diets which present the same appearance. In his analytic results Kosterlitz has shown that the cytoplasm as a whole suffers a decrease of substance in the protein-depleted state, and he has coined the expression "hypocyttoplasmic liver" to describe this entity.

Figure 2 illustrates the histologic appearance when a fourteen day period of a nonprotein diet was followed by four days of starvation. The vacuolation of the

3 Brues, A. M., Drury, D. R., and Brues, M. C. *Arch. Path.* **22**: 658, 1936.

4 Elman, R., and Heifetz, C. J. *J. Exper. Med.* **73**: 417, 1941.

5 Kosterlitz, H. W. *J. Physiol.* **106**: 194, 1947.

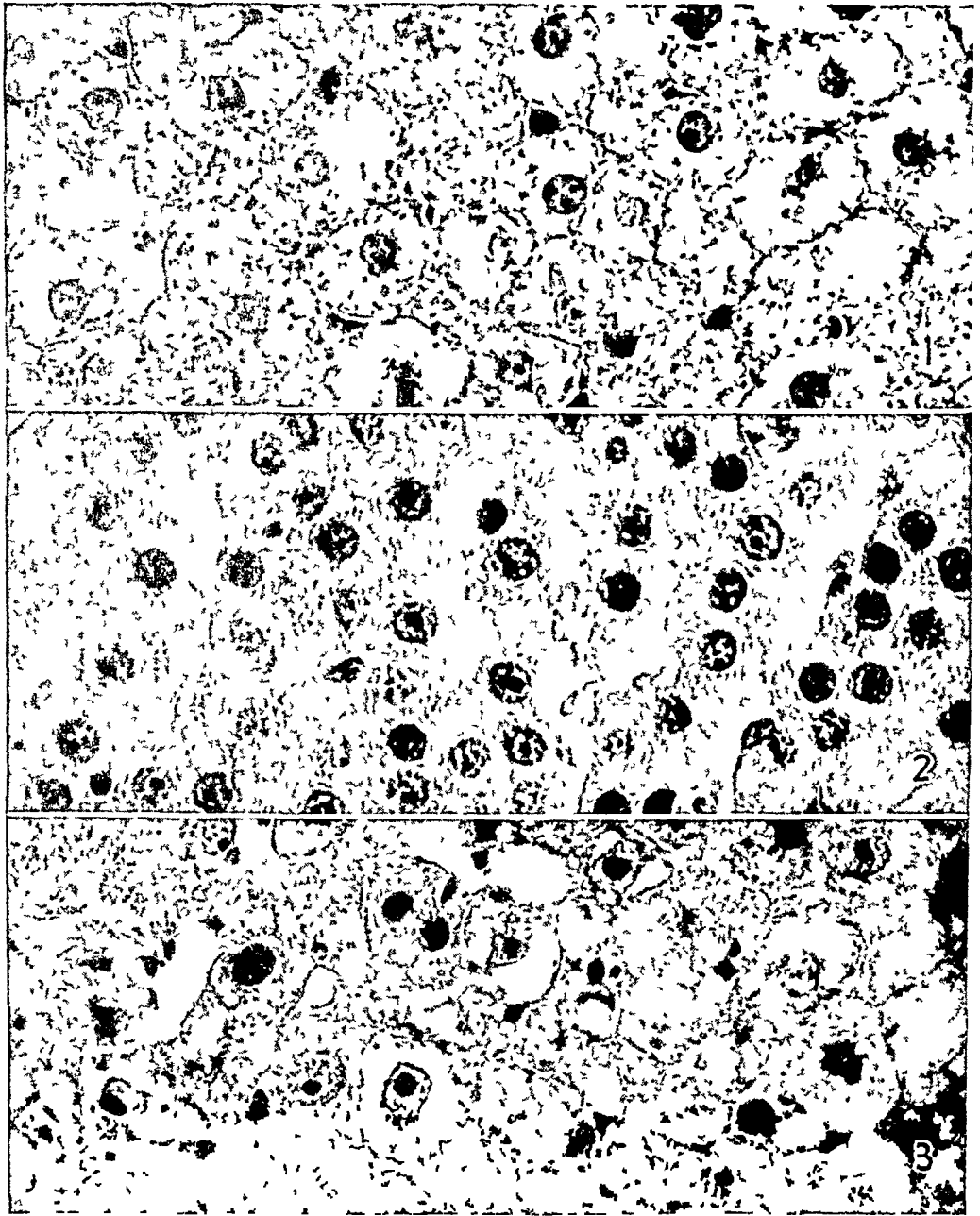


Fig 1—Section of liver (31A) of a rat fed a nonprotein diet for fourteen days but not fasted, $\times 800$ Large cells, markedly vacuolated, are seen showing shreds of eosinophilic cytoplasm but no basophilic intracytoplasmic particles

Fig 2—Section of liver (228B) of a rat which had undergone fourteen days of a nonprotein diet followed by four days of starvation, $\times 800$ Note the reduction in the size of the cells as compared with those in figure 1, also, the loss of vacuoles and the smooth texture of the cytoplasm, without large granules

Fig 3—Section (31B) from the same liver as that shown in figure 1, taken twenty-four hours after partial hepatectomy, $\times 800$ Cells of varying size are seen, with marked vacuolation in some and evidence of active mitosis

cytoplasm disappeared. Cytoplasmic particles were still absent, and the cells were much decreased in size.

The terminal histologic aspect of the liver during the first two days after operation is illustrated in figure 3. There was usually some increase in stainable lipid in addition to active mitosis. However, by analysis the lipid concentration never exceeded 9 per cent in either series 1 or series 2. Postoperative feeding of adequate amounts of protein^{1b} led to recovery of the staining properties and reappearance of cytoplasmic particles as illustrated in figure 4. Two weeks after operation the regenerated liver tissue differed from that of controls not operated on in two respects. First, the naked eye could perceive the increase in size of the regenerated lobules on either the serosal or the cut surface of the liver. Second, the organ was grossly soft and friable, and microscopically the supportive stroma was sparse and immature.

Series 2 of table 1 shows the outcome of 69.4 per cent hepatectomy in 144 rats prepared for operation by being restricted to a nonprotein diet for fourteen

TABLE 2—Pathologic Observations in Livers of 6 Rats Showing Massive Necrosis

Rat	Post operative Survival, Hr	Time of Necropsy	Gross Observations	Microscopic Observations				Leuko- cytic Infiltration
				Necrosis	Hemor- rhage	Inclusion Bodies	Mitosis	
98	26	<5 hr	Pale, yellow, with large hemorrhages	++++	++++	++++	0	±
116	76	<1 hr	Moderately pale, with small hemorrhages	++	+	0	0	+
126	47	2 min	Pale, yellow, with small hemorrhages	++	+	++++	±	++
135	29	*	Pale, gray, with large hemorrhages	++++	++++	++++	0	±
173	20	30 min	Pale, gray, with large hemorrhages	++++	+++	++++	0	±
273	24	3 min	Pale, yellow, with multiple small hemorrhages	++++	++++	++++	0	+++

* This rat was killed while moribund.

days. Three of these died at operation, while 15 died subsequently. Ten of the 15 which succumbed in the postoperative period had degenerative changes in the liver. Six of these had hemorrhagic necrosis of the liver.

The observations on these 6 animals are analyzed in table 2. The postoperative course was similar in each case. All animals recovered from the immediate effects of the operation and were generally walking about for several hours after recovery from the anesthetic. They were uniformly anorectic and ate nothing between operation and death. During the second or third twelve hour period they became progressively less active. Respirations became rapid, and before death they were generally quite unresponsive to stimulation. In an occasional animal terminal convulsions of a generalized clonic type developed. Death usually occurred, as shown in table 2, during the second day. The timing and the pattern of events were quite similar to the so-called "liver death" of clinical experience.⁶

6 Boyce, F. F. The Role of the Liver in Surgery, Springfield, Ill., Charles C. Thomas, Publisher, 1941.

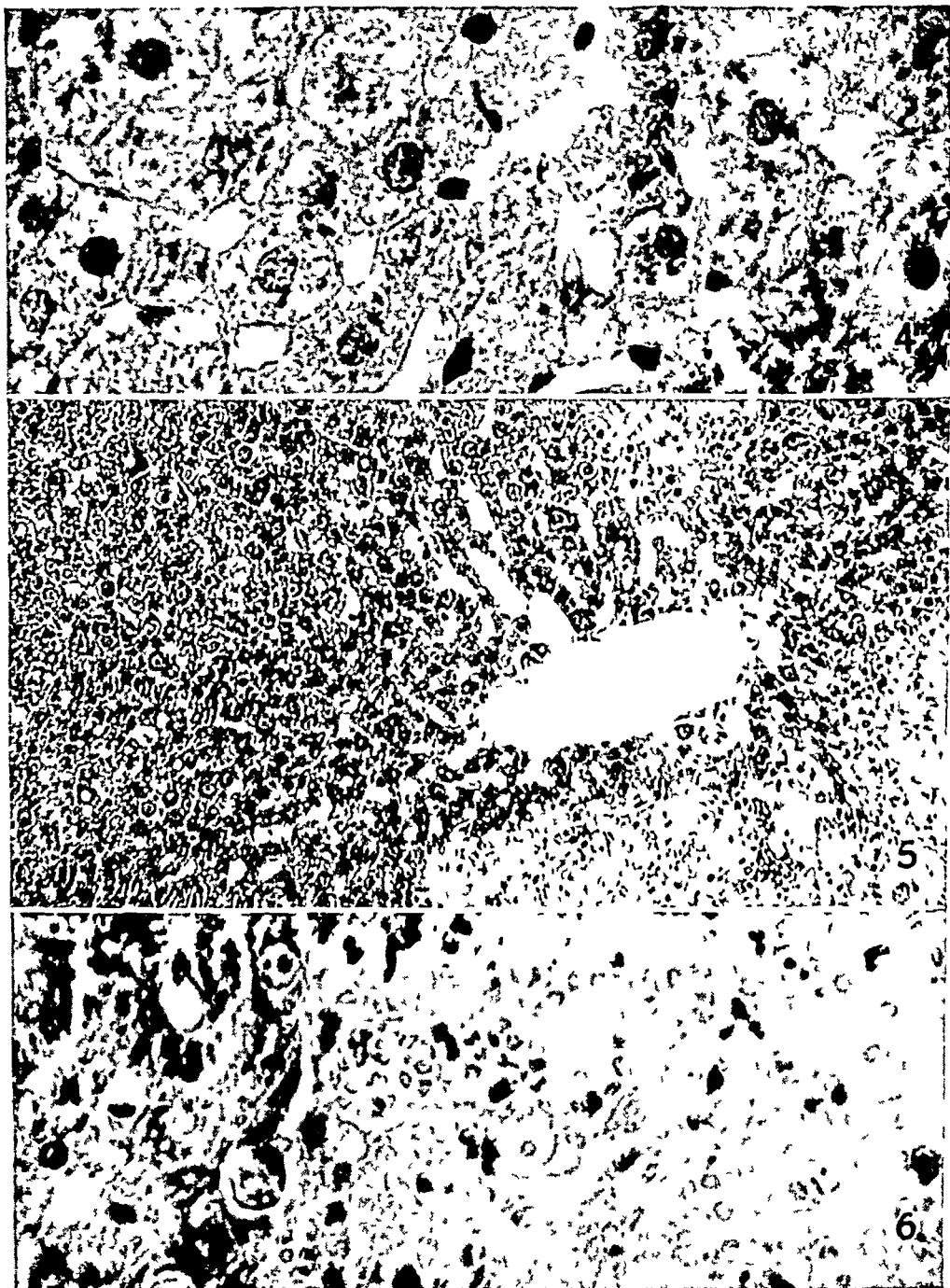


Fig 4—Section of liver (127B) of a rat which was fed a nonprotein diet for fourteen days, then partially hepatectomized and finally fed a diet containing 18 per cent casein for fourteen days after operation. The appearance is that of liver of a normal protein-fed rat. The cells are large, the cytoplasm stains heavily and contains an abundance of basophilic granules.

Fig 5—Liver of rat 273 (table 2), whose death occurred twenty-four hours after operation, with autopsy being made at once. Giemsa stain, $\times 150$. Areas of massive necrosis are seen, with a relatively intact collar of liver tissue about the central vein.

Fig 6—Portion of section shown in figure 5, Giemsa stain, $\times 700$. Details of the necrotic area may be seen. Several inclusion bodies are evident in a corner of the section.

The necrotic livers described in table 2 were grossly pale except for the flame-shaped hemorrhagic areas, which appeared to involve indiscriminately all areas of the liver remnant

The histologic picture was the same in all cases, differences being only of degree. Figures 5 and 6 show a characteristic section under low and high magnification. The massive character of the necrotic lesion is evident. Much of the necrotic parenchyma is involved by infarction due to presence of blood. The degree of leukocytic infiltration of the infarcts varied from animal to animal. The lesions appeared to be midzonal in origin, and often the only intact parenchyma in a lobule was a collar about a central vein.

Vacuoles containing spherical inclusions were prominent in all but 1 liver. These were eosinophilic and hyaline. The smaller of these bodies were distinctly intracellular and showed no particulate structures. Many larger ones appeared to lie outside the liver cells and to contain red blood cells. It was impossible to determine unequivocally whether some were formed outside the liver cells, or whether all originated in the cytoplasm of the parenchyma and later broke out of the cells as the latter disintegrated. In 5 of the 6 livers mitotic figures could not be found even in undamaged parenchyma, in the sixth they were extremely rare. Histologically, fat was always present in the uninvolved tissue but not always in large amount.

Four other rats in series 2 showed at death a focal degenerative lesion of the liver without hemorrhagic infarction of a type which might be classified as focal coagulation necrosis. Small, indiscriminately scattered areas of pale, degenerated cells, showing no nuclear material, characterized this lesion. Inclusion bodies were not seen. These deaths occurred later than those in the cases of hemorrhagic necrosis, from two to seven days after operation. Mitoses were few but were not so conspicuously absent as in the hemorrhagic livers.

Series 3 of table 1 consisted of a group depleted of protein for twenty-eight days before operation and restricted to the nonprotein diet for eight days post-operatively. No hepatic lesions were found in this series, and there was no notable loss of the ability of the liver to regenerate^{1a}. Series 4 received sodium ricinoleate, 0.5 cc. of a 10 per cent solution per one hundred grams of body weight being injected subcutaneously at the time of operation. This treatment superimposed a sterile abscess on the stress of depletion and partial hepatectomy. The animals were more severely affected than by the operation alone. Two of the 6 died within thirty-six hours with evidence of focal degenerative changes in the liver but no extensive necrosis.

Series 5 and 6 were submitted to an operation in which not only the median and right lateral lobes but also the caudate lobe was removed². The operation was found to involve approximately 77 per cent removal of the liver. The procedure did not disturb the well nourished animals in series 5 but was definitely a most severe strain on the protein-depleted rats. In series 6 all animals were seriously ill, all had bile pigments in the urine and an unusual amount of extravasated blood in the laparotomy wounds. Eleven animals survived operation, 4 of which died after between sixteen and forty-eight hours. Focal degenerative lesions were observed in 2. One survivor which appeared to be recovering well when killed at forty-eight hours provided a unique histologic observation: a liver involved by moderately extensive hemorrhagic necrosis with extremely active mitosis in the intact tissue. This is the only instance suggesting that an animal can recover from an established hemorrhagic necrosis of this type.

LIVER PROTEIN REGENERATION IN NECROTIC LIVERS

Three of the livers shown in table 2 were analyzed for protein by the methods previously described^{1a} Rat 116 had a postoperative liver protein increment of 0.08 Gm per hundred grams of initial body weight The expected increment at seventy-six hours had been found from previous experiments to be between 0.10 and 0.14 Gm Rat 126 showed an increment of 0.07 Gm against an expected figure of 0.10 Gm at forty-eight hours Rat 135 had gained 0.10 Gm in twenty-nine hours, which exceeded the expected figure of 0.04 Gm, but this liver, unlike the other 2 livers, was heavily infiltrated with blood The findings in rats 116 and 126, in which the analytic picture was not greatly obscured with bloody infiltration, provide a slender correlation with the apparent suppression of mitotic activity observed in the histologic sections It is suggested that the necrosis and death were related to a failure of the organ to initiate a prompt regenerative response

No evidence was found of an increase of extractive (nonprotein) nitrogen in these livers, which is in conformity with the findings of Himsworth⁷ and Glynn⁷ in their dietetic necrosis Fat and glycogen analyses were not done on any of the individual animals listed in table 2

PATHOLOGIC CHANGES IN OTHER SITES

Thymus—This organ was not weighed at autopsy but was examined grossly in every case and frequently histologically Gross observation showed that it decreased in size during a fourteen day period of a nonprotein diet, although even six weeks on this diet failed to cause extreme atrophy However, in the animals depleted of protein for fourteen days and then partially hepatectomized, a sudden and extreme reduction in the size of the gland was found to occur Within forty-eight hours after operation it was often difficult to identify the organ at all, and sections showed an extreme reduction in the size and the number of lymphoid follicles, with considerable reticulum which was virtually empty of lymphoid cells This condition was found to persist up to fourteen days after operation unless the animal was realimented on a protein-containing diet The question is raised whether lymphoid tissue, as exemplified by the thymus, may not contribute to the internal shifting of body nitrogen to the liver, which must occur when the latter organ undergoes regeneration in the absence of exogenous protein⁸

Kidneys—No hemorrhagic or degenerative lesions were noted in the kidneys Many protein-depleted animals showed mottled yellow areas on the surface of the organ and extending part way through the cortex These areas were especially marked in series 6, which were the most severely affected of any animals With fat stains such areas were found to contain pathologic amounts of fat, localized in the cells of the proximal convoluted tubules

Adrenal Glands—No lesions were found under any circumstances tested The only groups which showed any gross increase in the size of the adrenal glands were those subjected postoperatively either to total starvation or to restriction of their caloric intake The adrenal glands of animals fed the nonprotein diet were small and appeared to remain so after the operation In general, the glands of rats in a good nutritional state were larger than those of animals which were protein depleted, but there was no gross evidence of hypertrophy of the adrenal glands after the operation, nor of any change in the microscopic appearance

7 Himsworth, H P, and Glynn, L E Biochem J **39** 267, 1945

8 White A, and Daugherty, T F Endocrinology **41** 230, 1947

Laparotomy Wounds—In the majority of the cases in which a rat was killed sections of the site of the laparotomy wound were made at the time of death. Little can be said about differences in healing which might be attributed to the various diets. Even in animals fed a nonprotein diet throughout the experiment the wound appeared to heal as well as in those which were adequately fed. So far as could be seen with hematoxylin and eosin staining, the only difference between the two groups was an appearance of greater cellularity in the fibrous scars of the protein-depleted animals. There was no difference in the incidence of wound infection. No evisceration occurred.

COMMENT

The necrotic lesions observed after partial hepatectomy in the livers of a small percentage of our animals are the first of their type to be reported. They occurred only in rats which were partially hepatectomized while in a state of partial protein depletion. It is considered that the necrosis appearing in the liver remnant is related in some way to the protein-depleted state.

It has been shown previously that a liver low in protein is especially susceptible to necrosis following chloroform anesthesia⁹. The same observation applies to the liver of an animal in which the protein stores have been decreased¹⁰. In our rats we have found that the fourteen day preoperative period of a nonprotein diet decreased the liver protein by approximately 40 per cent^{1a}. Accordingly, the standard surgical ablation of 70 per cent of the remaining liver resulted in a considerably greater net reduction of liver protein than was found when the same operation was performed on well nourished animals. It is possible that the severe reduction of liver substance achieved in the protein-depleted rats created a set of circumstances in which a "spontaneous" type of hepatic necrosis might occasionally make its appearance.

The basic causation of the necrosis is not explained by the related protein depletion. It is quite likely that the extreme attenuation of liver function which must have occurred in our animals led to a vicious cycle of events which ended in the destruction of an organ usually capable of maintaining at least structural integrity up to the time of death. For example, it could be postulated that suppression of the formation of prothrombin might have contributed to the hemorrhage of the liver's own parenchyma. Infection might well have played a part in the necrosis. Indeed, the lesion bears a resemblance to the focal necrosis described by earlier authors as occurring in overwhelming infections, especially in enteric diseases such as typhoid¹¹. It is emphasized that we have no direct evidence as to the part played by prothrombin.

9 Goldschmidt, S., Vars, H. M., and Ravdin, I. S. *J. Clin. Investigation* **18** 277, 1939.

10 Miller, L. L., and Whipple, G. H. *Am. J. M. Sc.* **199** 204, 1940.

11 Mallory, F. B. *J. M. Research* **6** 264, 1901.

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deficiency, infection or any other specific factor in the causation of the lesion

The finding of eosinophilic inclusion bodies suggests a virus infection. However, it is known that inclusions are not necessarily a definite proof of virus disease, for they are also found in necrosis of the liver after tannic acid treatment of burns¹² and in high altitude anoxia and in carbon monoxide poisoning¹³. There is every likelihood that the appearance of inclusion bodies may represent a phase of physicochemical disintegration of the dying cell without specific etiologic significance.

The lesion described by Glynn and Himsworth¹⁴ differs from ours in that the necrotic foci were not characterized by hemorrhage. Nevertheless, the two lesions appear to be initiated by a dietary insufficiency, specifically of protein or a component of protein.

Two facts in connection with the hepatic necrosis and death of the protein-depleted animals appear to be demonstrated by our observations. First, the animals were in a state of precarious compensation as to liver function for about forty-eight hours after operation. That is, the attenuation of liver substance was near the minimum compatible with survival. This impression was tested further with the experiment shown in series 6 of table 1, in which 12 protein-depleted rats were subjected to 77 per cent hepatectomy. Evidences of liver failure were abundant in this series, namely, the increased lassitude and anorexia, the bile pigments constantly appearing in the urine, the increased bleeding tendency and the higher mortality.

The second fact is that there was no mitotic activity in the necrotic livers detailed in table 2. The fragmentary analytic data already described for 3 of these animals support the conclusion that death was related to a failure of the hyperplastic regenerative response. It may be possible that the level of liver function left to the animals of our standard protein-depleted series was not only near the minimum required for survival but actually below that minimum and that survival was conditional on a certain degree of regeneration being successfully effected within about forty-eight hours. It is probable that in the first hours after operation the liver remnant fell behind in the performance of its essential metabolic work but that in the majority of animals it increased its capacity through regeneration in time to catch up with the immediate demands before the consequences of deficiency became irreversible. In the animals in which the regenerative effort was inadequate, death occurred about the second day.

12 Belt, T. H. *J. Path. & Bact.* **48**: 493, 1939. Duffin, J. D. *Canad. M. A. J.* **47**: 138, 1942.

13 Kritzer, R. A. *War Med.* **6**: 369, 1944.

14 Glynn, L. E., and Himsworth, H. P. *J. Path. & Bact.* **56**: 297, 1944.

We are not in a position to present studies of metabolites circulating in the blood to support this interpretation. We feel, however, that the observations are of interest and that the protein-depleted preparation described is of potential usefulness in the experimental study of hepatic insufficiency.

SUMMARY

Pathologic changes observed in the livers of 230 rats which were submitted to partial hepatectomy are reported.

A massive hemorrhagic necrosis of the liver is described, which occurred during the second day after operation in animals which had been previously partially depleted of protein through being restricted to a nonprotein diet for fourteen days.

The livers involved in this necrosis showed failure of the usual regenerative response. Mitotic activity was conspicuously absent, and fragmentary analyses suggested that the regeneration of new liver protein was retarded.

Evidence has been discussed to support the hypothesis that the functional liver tissue remaining in the protein-depleted rats after 70 per cent hepatectomy was inadequate to support life and that survival was conditional on a prompt regenerative response.

Some observations on the gross and histologic changes in the thymus, the kidneys, the adrenal glands and the laparotomy wounds are also included. A rapid involution of the thymus was noted in protein-depleted rats after partial hepatectomy.

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UNUSUAL HAMARTOMA OF THE LUNG IN A NEWBORN INFANT

CYRIL J. JONES, M.D.

MONTREAL, CANADA

THE TERM "hamartoma" was coined in 1904 by Albrecht,¹ who defined it as comprising "tumor-like malformations in which occur only an abnormal mixing of the normal components of the organ. The abnormality may take the form of a change in quantity, arrangement or degree of differentiation, or may comprise all three. The deduction to be drawn from histologic examination of these formations is that they have originated in abnormal mixing of their development." In the cases that he described the lesion was in the liver, and he did not describe the type of pulmonary malformation under discussion. However, since 1904, pulmonary lesions which had previously been referred to by a variety of names but which met the criteria of Albrecht's definition began to be increasingly termed hamartoma.

Hamartoma of the lung is uncommon. The subject has been reviewed by Hickey and Simpson,² Verga,³ McDonald, Harrington and Clagett,⁴ and Simon.⁵ In 1944 McDonald and associates⁴ collected 73 cases of the chondromatous type of hamartoma of the lung reported up to that time and added 23 similar cases of their own. From 1944 to the present 11 cases of chondromatous hamartoma of the lung have been reported.⁶ Simon,⁵ in a recent review of hamartoma of the lung has collected 25 cases of the hemangiomatous type. This brings the total number of reported cases of hamartoma of the lung to 132, of

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1 Albrecht, E. *Verhandl. d. deutsch. path. Gesellsch.* **7**: 153, 1904.

2 Hickey, P. M., and Simpson, W. H. *Acta radiol.* **5**: 475, 1926.

3 Verga, P. *Pathologica* **24**: 1, 1932.

4 McDonald, J. R., Harrington, J. W., and Clagett, O. T. *J. Thoracic Surg.* **14**: 128, 1945.

5 Simon, M. A. *Am. J. M. Sc.* **216**: 33, 1948.

6 (a) Cid, J. M. *Arch. Soc. argent. de anat. norm. y pat.* **2**: 199, 1940, cited by Itoiz.^{6e} (b) Bianchi, A. E., and Etchegoyen, H. *An. Inst. modelo de clin. med.* **24**: 405, 1943. (c) Latienda, R. I. *Arch. Soc. argent. de anat. norm. y pat.* **8**: 73, 1946. (d) Moreno, G. *Bol. y trab. Acad. argent. de cir.* **30**: 526, 1946. (e) Itoiz, O. A., Latienda, R. I., and Murray, A. J. *Arch. Soc. argent. de anat. norm. y pat.* **8**: 223, 1946. (f) Sassaroli, S. *Ann. radiol. diag.* **16**: 415, 1942. (g) Simon, M. A., and Ballon, H. C. *J. Thoracic Surg.* **16**: 379, 1947.

which 107 appeared to represent an abnormal mixing of the elements of the bronchial wall and 25 apparently represented an abnormal development of the vascular components of the lung. Since this report is concerned with a case that is apparently related to the former group, no further reference to hemangioma of the lung need be made.

The so-called chondroma of the lung is reported as occurring in from 0.027 per cent^{6a} to 0.25 per cent⁴ of routine autopsy cases. Among 84 cases in which sex was indicated there were 61 males and 24 females. In the majority of instances the chondromatous tumor-like masses were incidental findings at autopsies. They were usually subpleural in location and in some instances had led to an erroneous roentgen diagnosis of Ghon tubercle. The lesions were usually small and ranged in size from a few millimeters to 3 or 4 cm. in diameter. However, McDonald and associates⁴ mentioned a case in which the mass occupied the entire pleural cavity. Only in relatively few of the cases reported in the literature did the tumor reach a size sufficient to cause symptoms requiring surgical intervention.⁷

Hamartomas of the so-called chondroma type are described in general as being dense, somewhat hard, lobulated, bluish gray to white and having on section the general appearance of a true chondroma, but microscopically islands of epithelium, fetal fat, fibrous tissue and other features are found which betray their true nature.

Cancerous changes of lesions of this type are apparently rare. Greenspan⁸ described a case of pulmonary osteochondrosarcoma and in his review suggested two possibilities as to the origin of the tumor. It was thought that it might have represented either a cancerous change of a preexisting hamartoma or that it was a true osteochondroma. Simon and Ballou^{6a} described local cancerous features in their case of hamartoma. It is perhaps of interest to mention the cases reported by Cid^{6a}. Of the 2 patients described, the first, a 78 year old man, died of pulmonary edema and a bronchiogenic cancer of the right cervical region. A small hamartoma was found in the lung. The second, a 56 year old man, died of a bronchiogenic carcinoma, which was located in the hilar region of the left lung. At autopsy a small hamartoma was also found in the subpleural region of the lower lobe of the left lung.

The ages of the entire series of 107 patients with chondromatous hamartoma reported in the literature range from 21 to 78 years. A hamartoma of the lung of unusual though somewhat similar type occurring in a newborn infant is therefore considered to be of sufficient interest to report.

7 McDonald and others⁴ Simon⁵ Sherwood, K., and Sherwood, H. *Journal-Lancet* 52: 395, 1932.

8 Greenspan, E. B. *Am. J. Cancer* 18: 603, 1933.

REPORT OF A CASE

A baby girl was born during the twenty-eighth week of pregnancy at 6 10 a m on March 8, 1948. She required extensive resuscitation, and only after twenty-five minutes of effort were spontaneous respiratory movements established, and these were irregular. In a short while these gradually diminished, and the infant died one hour after birth. The mother was a 21 year old primipara. The labor was of spontaneous onset and uncomplicated. The presentation was vertex.

Autopsy—The infant weighed 1,516 Gm and measured 52 cm crown-heel and 38 cm crown-rump. The heart weighed 11 Gm, and its epicardial surface



Fig 1—Hamartoma occupying almost the entire upper lobe of the right lung. A small portion of the middle lobe of the lung is also shown.

presented a few small petechial hemorrhages. The remaining organs, except for the lungs, were not remarkable.

The entire left lung weighed 15 Gm and was atelectatic but otherwise not remarkable. The right lung weighed 50 Gm and was partially aerated. The upper lobe (fig 1) was full, rubbery and firm in consistency, and the pleural surface was blotchy brown. On section this fulness in the upper lobe was seen to be due to a spherical, circumscribed but nonencapsulated mass, 3.5 cm in diameter, occupying almost the entire lobe, having a margin of compressed lung

tissue near the interlobar fissure. The cut surface was firm and pink with coarse whorls and interlacing bands of white fibrous tissue in which no cartilaginous component could be distinguished on gross examination. No large bronchi were present within the mass. The residual lung tissue of that lobe was collapsed.

Microscopic Examination—The normal structure of the upper lobe of the right lung was completely altered and replaced by wide interlacing bands or bundles of tissue, some of which were seen in cross section as islands (fig 2). They were composed of streaming lines of uniform elongated cells in parallel arrangement separated by sparsely distributed fibers. Their cytoplasm was thin, their nuclei,



Fig 2—A representative section of the hamartoma showing fibrous tissue trabeculae with dilated capillaries, islands of cartilage and bronchiolar structures interspersed through the fetal lung tissue $\times 24$

elongated, oval and vesicular. These cells resembled fibroblasts. The delicate thin fibers proved, in sections stained with Van Gieson's stain, to be collagen. Irregularly scattered throughout these bands were rounded, oval or irregularly shaped small islands of immature cartilage, and more rarely areas of fetal fat. Scattered here and there in the fibrous bands were channels lined by respiratory epithelium, but no lymphoid tissue was seen. The intervening lung tissue was fetal in type. Portions of the bronchiolar mucosa were plicated, and their walls were in apposition. There was marked congestion of the capillaries, and one small area of hemorrhage was present.

The cause of death appeared to be prematurity, complete atelectasis of the left lung and partial atelectasis of the right lung, complicated with a hamartoma of the upper lobe of the right lung

COMMENT

It is evident that the lesion in the upper lobe of the right lung in the case reported represents a hamartoma according to Albrecht's original definition, being in essence composed of masses of immature mesenchymal tissue, fibrous tissue, fetal fat, cartilage and imperfectly formed bronchiolar structures inextricably mixed up with more normally developed lung tissue. It presents none of the usual histologic features of a true tumor, and it is unquestionably benign in appearance. The small proportion of cartilage and much larger proportion of fibrous tissue elements set this hamartoma apart from the majority of those reported. In the latter, cartilage of fetal or of adult type usually composed the major portion of the malformations. In 3 exceptional cases of McDonald and associates⁴ the anomaly had no cartilage.

Whereas hamartoma has not hitherto been reported as occurring earlier than the age of 21 years, the expectation that it should be discoverable at any time after birth is inherent in the concept of the hamartomatous malformation. That cases have not been reported heretofore in which the earlier age groups were concerned seems extraordinary. In any event the present case emphasizes the possibility that hamartoma may present itself as a clinical problem even in the neonatal period. If recognized as the cause of serious clinical manifestations, it should be entirely amenable to surgical removal.

SUMMARY

An unusual hamartoma, of fibrochondromatous type, involving almost the entire upper lobe of the right lung of a newborn premature infant is presented.

ABSENCE OF THE PULMONARY ARTERY A NEW CLASSIFICATION FOR PULMONARY ARTERIES OF ANOMALOUS ORIGIN

Report of a Case of Absence of the Pulmonary Artery with Hypertrophied
Bronchial Arteries

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ANOMALIES of the pulmonary artery occur rather frequently in congenital heart disease. These have been classified as stenosis or atresia of the pulmonary artery (truncus solitarius aorticus), persistent truncus arteriosus communis or patent ductus arteriosus. There is, however, a group of anomalies which we believe cannot be properly classified under any of the diagnoses named. For this group we propose a new classification, absence of the pulmonary artery. We have such an anomaly to report, and we believe that a consideration of its genesis and diagnosis will justify the separation of this from the other anomalies of the pulmonary artery.

REPORT OF CASE

The patient was a 10 month old Negro boy who was born April 24, 1942 and was first seen in the infant welfare clinic at the age of 6 weeks. There were no complaints at that time, and examination was reported to show no significant abnormalities. Two months later rapid respiration was noted, and a systolic murmur was found over the entire precordium. A roentgenogram of the chest showed enlargement of the heart, which had a well rounded left ventricle, and widening of the vessels of the mediastinum (fig 1). The patient was followed at frequent intervals, and his condition remained unchanged. He died suddenly at home, Feb 22, 1943, and the only history obtainable at the time of autopsy was that he had shown symptoms of a "heavy cold". The abnormalities noted at autopsy aside from those of the heart were left lobar pneumonia with bilateral pleural effusion, acute splenic tumor with enlargement of the mesenteric and mediastinal lymph nodes, acute passive congestion of the liver, and a small congenital umbilical hernia.

The heart was normal in position but appeared moderately enlarged and weighed 95 Gm. The two sides of the heart were of equal size. The right atrium

received the venae cavae and the coronary sinus and emptied into the right ventricle through a normal tricuspid valve. The left atrium received the right and left pulmonary veins, of usual size, and emptied into the left ventricle through a normal bicuspid valve. The interatrial septum was intact. There was hypertrophy of both ventricles, relatively greater on the right, so that the ventricular cavities were of approximately equal size. The myocardium measured 9 mm in thickness at the base of the left ventricle and 8 mm at the base of the right ventricle. There was a large defect at the base of the interventricular septum, 1 cm in diameter, with the aorta overriding this defect, approximately one third to the left and two thirds to the right. The aorta was greatly dilated at its origin, its ring measuring

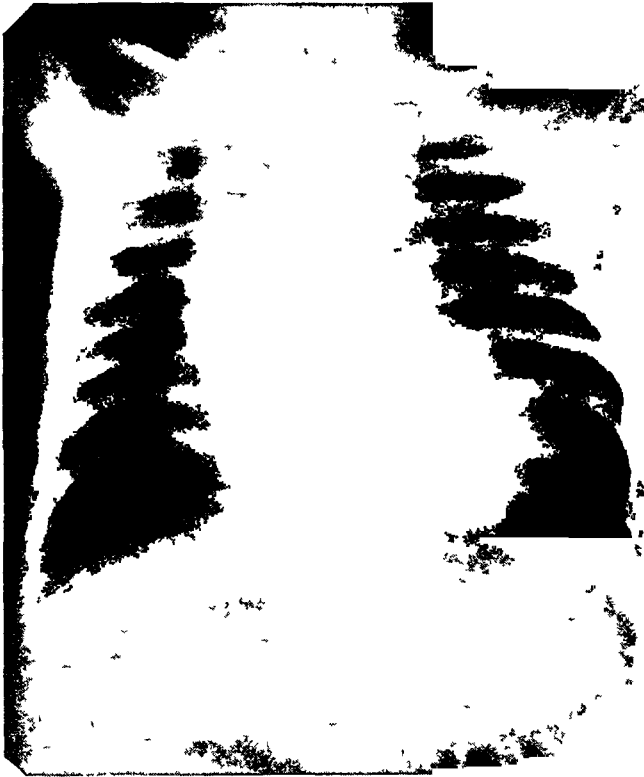


Fig 1—A roentgenogram showing cardiac enlargement, the typical “coeur en sabot” outline of the heart and remarkable widening of the mediastinal shadow. The hilar shadow is obscured on the right side but is normally prominent on the left.

5 cm in circumference, and it presented three normal valve cusps, the coronary arteries arising from the right and left anterior sinuses. There were no other vessels arising from the heart, and there was no evidence of even a rudimentary pulmonary valve. The pulmonary artery was not present, nor was there any other vessel or remnant of a vessel which could represent the pulmonary artery. The ductus arteriosus was absent. The aorta assumed its normal course and gave rise to its usual branches. The bronchial arteries arose in their normal position from the descending aorta but were tremendously dilated and hypertrophied, being of about the same size as the celiac artery. They entered the lungs in close association with the posterior walls of the bronchi and constituted the lungs' sole supply.

of arterial blood. Dissection of the hilus of each lung revealed that there were no other arteries entering the lung, and confirmatory evidence of this was obtained on microscopic examination of sections of the lung, which failed to show the presence of arteries other than the dilated bronchial arteries. Microscopic sections of the heart also showed hypertrophy and fragmentation of the muscle fibers.

The anatomic diagnosis of the cardiac defects follows: absence of the pulmonary artery, compensatory dilatation and hypertrophy of the bronchial arteries, dextroposition of the aorta ("rider" position), basal interventricular septal defect, dilatation of the ascending aorta, cardiac hypertrophy.

Figure 2 is a semidiagrammatic representation of the essential structural defects.

EMBRYOLOGIC CONSIDERATIONS

A consideration of the embryonal mechanism involved in the production of anomalies of the heart is essential to a correct interpretation and appreciation of the defects as seen at autopsy, and it is through

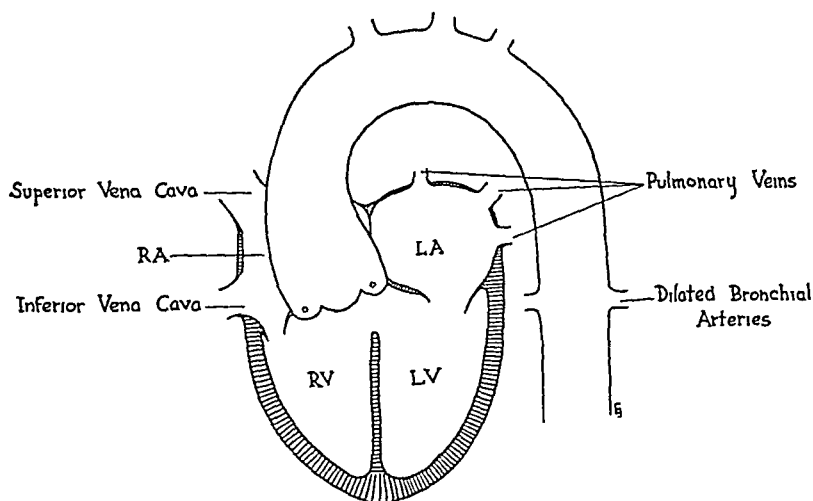


Fig 2—A diagrammatic representation of the heart showing absence of the pulmonary artery, hypertrophied bronchial arteries, dextroposition of the aorta and an interventricular defect.

such studies that the array of seemingly heterogeneous defects can be welded into pathologic and clinical entities. The fundamental lesion in our case was an absence or an early involution of the primitive sixth aortic arch, from which the normal pulmonary artery and ductus arteriosus develop. If the sixth arch had continued its usual development until the pulmonary artery was established, then any subsequent arrest of growth would have led to the more common stenosis or atresia of the pulmonary artery in which some remnant of the vessel is present. In cases of atresia the artery may be represented by no more than a thin fibrous band which may extend toward but not even reach the heart, as was found in one of our previous cases.¹

1 Manhoff, L. J., Jr., and Howe, J. S. *Am Heart J* 29:90, 1945.

The development of the pulmonary artery of the early embryo has been excellently described by Huntington,² and a knowledge of the essential steps involved will allow a better understanding of the various defects that result from arrested development at different stages. The first important consideration is that the pulmonary vascular bed is established independently of the sixth arch and is not dependent on that vessel for its development. The pulmonary plexus in its early stages is connected to the ventral branches of the dorsal aorta. As its growth progresses, it sends out a branch cranially which joins with the free extremity of the ventral root of the sixth aortic arch, and its connections

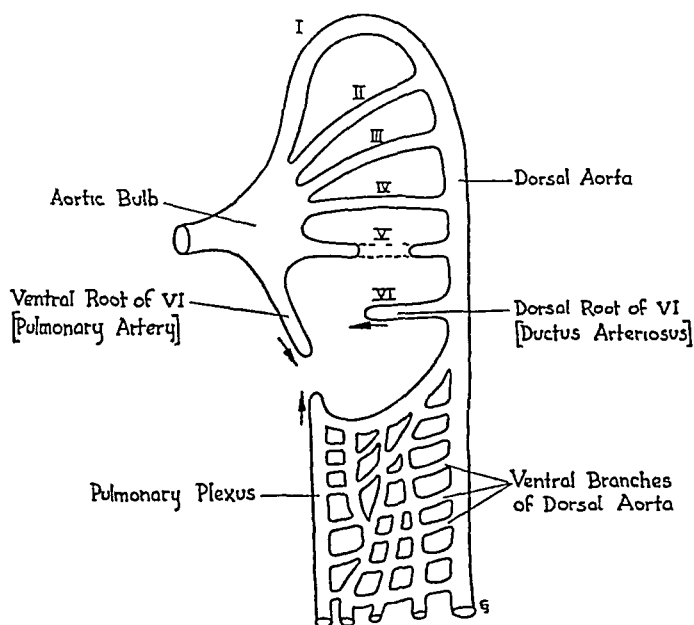


Fig 3—A diagram of the early stage of development of the pulmonary artery preceding the completion of the sixth arch (redrawn from Huntington²). Note that the pulmonary plexus develops independently of the sixth arch, and that it sends a vessel upward to join the ventral branch of the sixth arch. If the ventral branch were absent, this vessel could unite with the dorsal root, and if the entire sixth arch were absent the circulation in the pulmonary plexus could be maintained by the ventral branches of the aorta (usually the bronchial arteries).

with the dorsal aorta are later lost (fig 3). The dorsal root of the sixth arch joins the ventral root just distal to the aortic bulb, thus establishing a complete arch but leaving a free extremity of the ventral root to be joined by the cranial branch of the pulmonary plexus to complete the pulmonary artery. The dorsal root of the left side persists along with the left fourth arch (which forms the normal left arch of the aorta) as the ductus arteriosus, while the right dorsal root disappears along with the right fourth arch. The aorticopulmonary septum arises

from the angle between the fourth and sixth arches and grows toward the heart, dividing the common truncus arteriosus into the separate aorta and the main trunk of the pulmonary artery

Depending on the stage at which this process is interrupted, several variations of the pulmonary artery are possible. Impaired growth of the established pulmonary artery will lead to stenosis or atresia of the artery, as already mentioned, leaving a recognizable remnant of the vessel. Incomplete development of the aortopulmonary septum will fail to separate the trunks of the aorta and the pulmonary arteries, resulting in a persistent truncus arteriosus communis. If there is failure of the ventral root of the sixth arch, the dorsal root (future ductus arteriosus) may establish or maintain continuity with the pulmonary plexus and the pulmonary artery will then appear to rise from the arch of the aorta. If there is absence of the entire sixth arch, the pulmonary plexus will maintain its original connections with the ventral branches of the dorsal aorta and the lungs will be supplied through bronchial or other arteries. The aforementioned developmental failures may be associated with other mechanisms, such as persistence of normally obliterated parts of the dorsal aorta, persistence of parts connecting the pulmonary plexus with more caudal ventral branches of the aorta, or other secondary changes, so as to give rise to various anomalous vessels supplying the lungs.

ANALYSIS OF CASES

There is some disagreement in the literature about the proper classification and diagnosis of certain types of cases, namely those in which there is only a single arterial trunk arising from the base of the heart. In those cases in which the single trunk is a pulmonary artery with an atretic aorta (*truncus solitarius pulmonalis*), or an aorta with an atretic pulmonary artery (*truncus solitarius aoticus*) little difficulty is encountered, as these anomalies are easily recognized. Consequently we may eliminate the atresias from our consideration.

When the question arises whether the single trunk is a persistent truncus communis or simply a normal aorta with an aberrant pulmonary artery there is considerable indecision. There are at present no adequate criteria and no suitable nomenclature to settle this question. Identical anomalies have been reported variously as persistent truncus arteriosus communis, *truncus solitarius aoticus* or atresia of the pulmonary artery. Some clarification of these anomalies can probably be achieved by considering them with respect to the developmental defect of the pulmonary artery. For purposes of analysis, the cases may be divided into four groups according to the sites of origin of the pulmonary vessels (fig 4).

Group 1 Cases in which the lungs are supplied by arteries arising from the ascending aorta

Group 2 Cases in which the lungs are supplied by arteries arising from the arch of the aorta

Group 3 Cases in which the lungs are supplied by arteries arising from the descending aorta

Group 4 Cases in which the lungs are supplied by arteries having other anomalous origins

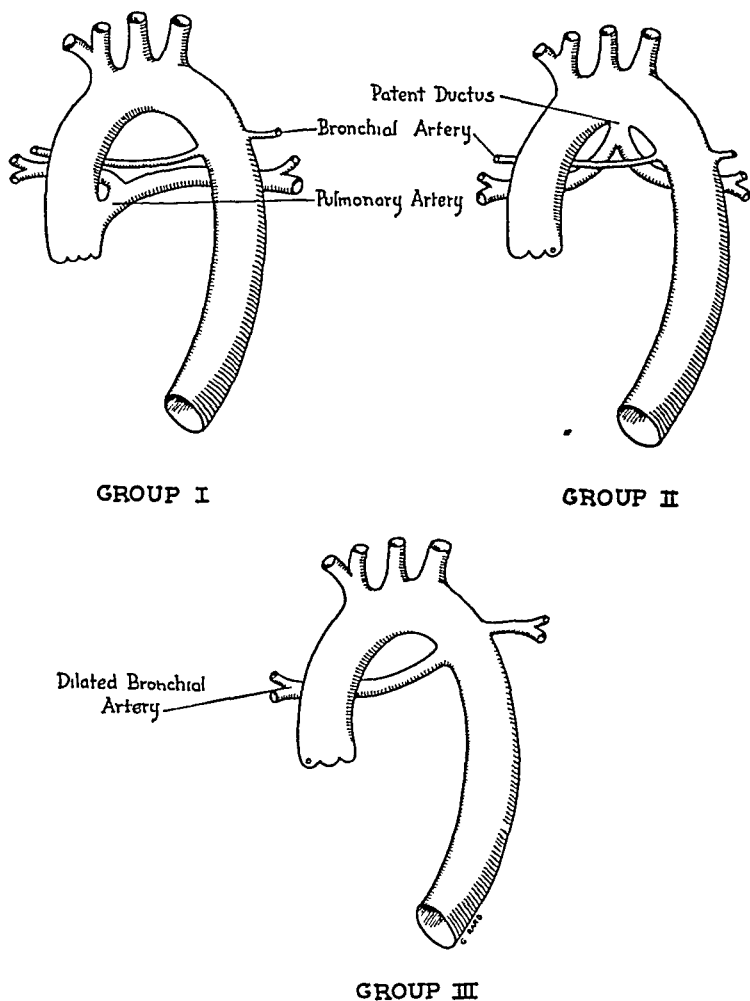


Fig 4—*Group 1* The otherwise normal pulmonary artery arises from the ascending aorta (persistent truncus arteriosus communis) *Group 2* The lungs are supplied by an artery arising from the aortic arch (absence of the pulmonary artery with persistent ductus arteriosus) *Group 3* The lungs are supplied by arteries arising from the descending aorta (absence of the pulmonary artery with hypertrophied bronchial arteries) Notice that in groups 1 and 2 there are two sets of arteries entering the hili of the lungs, while in group 3 there is only one, the bronchial arteries *Group 4* (not shown) a heterogeneous group similar to group 3 except that the lungs are supplied by various anomalous vessels

Group 1 These cases present little difficulty, as they are generally accepted as exemplifying persistent truncus arteriosus communis The arteries supplying the lungs are normal pulmonary arteries derived

from the sixth arch. A lack of development of the aorticopulmonary septum has merely failed to separate the root of the pulmonary artery from the aorta, so that both vessels arise from a common trunk, the primitive truncus arteriosus.

Group 2. These cases have usually been reported as instances of persistent truncus arteriosus communis (Graham and Montgomery,³ Hunter,⁴ Popjak⁵ and others), but we believe that this is an incorrect designation. The artery supplying the lungs is actually a persistent ductus arteriosus, while the true pulmonary artery is lacking. This results from an absence or an early involution of the ventral roots of the sixth arch (the true pulmonary artery), with the left dorsal root of the sixth arch (the ductus arteriosus) maintaining continuity with the pulmonary plexus and persisting as the sole arterial supply to the lungs. To consider this vessel arising from the arch of the aorta a true pulmonary artery, as has been suggested by some, would require two improbabilities. First, it could result from a failure of the aorticopulmonary septum, which is unlikely, as the cranial extent of this septum is at the junction of the embryonic fourth and sixth aortic arches, which is proximal to the adult aortic arch. A complete failure of the septum would thus result in a pulmonary artery arising from the upper end of the ascending aorta, and not from the arch. Second, it would require that a vessel arising from the sixth arch be transposed along the aorta cephalad to the origins of other vessels (innominate and common carotid arteries) which develop from the third and fourth arches. It is improbable that such a transposition of the primitive aortic arches could occur. Hulse⁶ stated that a true pulmonary artery must arise proximal to the innominate artery, and this seems to be a sound interpretation.

In some cases there may be more than one vessel arising from the concavity of the arch and supplying the lungs, and this could easily result from an absorption of the short trunk of the ductus arteriosus so that its branches would arise directly from the aorta. Another possibility that has been suggested is that these multiple vessels may represent migrated bronchial arteries. This could readily be determined, if it is a fact at all, by a careful examination of the hili of the lungs. If there is only one set of arteries entering the hili of the lungs, in close association with the bronchi on their posterior surfaces, then these would be bronchial arteries and the conception of misplaced bronchial arteries arising from the aortic arch would

3 Graham, S, and Montgomery, G. L. *J. Tech. Methods* **18** 97, 1938.

4 Hunter, O. B., Jr. *Arch. Path.* **37** 328, 1944.

5 Popjak, G. *J. Path. & Bact.* **54** 67, 1942.

6 Hulse, W. *Virchows Arch. f. path. Anat.* **225** 16, 1918.

have to be accepted. However, if two sets of vessels are found, then one would be the normal bronchial arteries and the other would represent the branches of the ductus arteriosus.

Group 3 is represented by our case and consists of hypertrophied bronchial arteries supplying the lungs in the absence of any form of the pulmonary artery. This results from an early involution or an absence of the entire sixth aortic arch. The pulmonary plexus then maintains its original communication with the ventral branches of the dorsal aorta, which probably give rise to the hypertrophied bronchial arteries. That this anomaly must result from an absence of the sixth arch rather than from a lack of development of the aortopulmonary septum seems obvious. As stated in a foregoing paragraph, even a complete failure of development of the septum would result in otherwise normal pulmonary arteries arising from the ascending aorta. It could in no way explain how arteries could originate from the descending aorta or from the arch of the aorta, nor could it explain absence of the pulmonary arteries.

Group 4 comprises a heterogeneous collection of rare types in which the arteries supplying the lungs arise from vessels not ordinarily associated with the pulmonary circulation. This includes cases in which the arteries arise from such vessels as the subclavian, intercostal, esophageal, phrenic, common carotid or inferior thyroid arteries⁷ or from the lower thoracic or the abdominal aorta or the celiac or the superior mesenteric arteries.⁸ A detailed discussion of their embryonal formation will not be attempted here, as each of these defects deserves individual consideration, and one who is interested will profit by a study of the separate case reports. However, in general, these anomalies may be considered as variations of the fundamental developmental defects described in groups 2 and 3. Those cases in which branches from the lower thoracic or the abdominal aorta are concerned are similar to those of group 3, the only difference being that the connections of the pulmonary plexus and the dorsal aorta are maintained through ventral branches lower down in the thoracic or the abdominal region of the aorta. In the other cases

7 (a) Abbott, M. E. Congenital Heart Disease, in Osler, W., and McCrea, T. Modern Medicine, Philadelphia, Lea & Febiger, 1927, vol. 4, p. 612, Congenital Heart Disease, in Nelson's Loose Leaf Living Medicine, New York, Thos. Nelson & Sons, 1931, vol. 4, p. 207, Atlas of Congenital Heart Disease, New York, American Heart Association, 1936. (b) Wheeler, D., and Abbott, M. E. *Canad. M. A. J.* **19**: 297, 1928. (c) Blount, R. F., and Poth, E. J. To be published, abstracted, *Anat. Rec.* **91**: 5, 1945. (d) McCotter, R. E. *ibid.* **4**: 291, 1910. (e) Batts, M., Jr. *J. Thoracic Surg.* **8**: 565, 1939. (f) Park, E. A. *Proc. New York Path. Soc.* **12**: 88, 1912. (g) Harris, H. A., and Lewis, I. *J. Thoracic Surg.* **9**: 666, 1940.

8 McCotter^{7d} Batts^{7e} Park^{7f} Harris and Lewis^{7g}

the anomalies have a more complicated origin, but their development is also based on the primary loss, either complete or partial, of the pulmonary artery

SUGGESTED CLASSIFICATION

From the foregoing, developmental considerations a new classification may be evolved for the congenital defects resulting from anomalous origins of the pulmonary blood supply. It is believed that it will aid in the proper interpretation and recognition of some of these heretofore controversial cases. Each of the previously discussed anomalies may be classified under one of the following heads.

Persistent Truncus Arteriosus The otherwise normal pulmonary arteries arise from the ascending aorta proximal to the origin of the innominate artery (group 1). Two sets of arteries enter the hilum of the lungs, the pulmonary arteries and the bronchial arteries.

Absence of the Pulmonary Artery with Persistent Ductus Arteriosus The lungs are supplied by the ductus arteriosus arising from the concavity of the arch of the aorta (group 2). It may arise by a single trunk or its branches may arise independently from the aorta. Two sets of arteries enter the lung hilum, the bronchial arteries and the branches of the ductus arteriosus.

Absence of the Pulmonary Artery with Hypertrophied Bronchial Arteries The bronchial arteries arise from the descending aorta (or possibly from the arch) and are considerably hypertrophied and dilated, constituting the sole arterial supply to the lungs (group 3). No other arteries enter the hilum of the lungs.

Absence of the Pulmonary Artery with Anomalous Pulmonary Vessels The lungs are supplied by arteries arising from other vessels not ordinarily associated with the pulmonary circulation (group 4). Two sets of arteries enter the hilum of the lungs, the bronchial arteries and the anomalous pulmonary arteries.

Isolated cases may show a mixture of these anomalies, the lungs being supplied by different anomalous vessels on the two sides, or there may be a normal pulmonary artery on one side with an anomalous vessel on the other side.

It should also be pointed out that these same anomalies may be associated with atresia rather than absence of the pulmonary artery, and thus the same classification may be used for cases of the latter type by merely substituting the word "atresia" for "absence". The functional and clinical significance is similar in either event, though the difference in the embryonal development of the anomaly should be kept in mind.

The term "absence of the pulmonary artery" was selected to identify these cases not only because it accurately defines both the anatomic lesion and the underlying developmental defect but also

because the terms in present use are inadequate to differentiate these cases. Truncus solitarius aorticus would partially describe the anatomic defect but would fail to differentiate between absence and atresia of the pulmonary artery. Stenosis or atresia of the pulmonary artery cannot, of course, be correctly applied in the cases in which there is an absence of the artery. The designation "persistent truncus arteriosus" has been used to include these cases, but we believe that it is not only incorrect but that its continued use hinders the proper understanding of these defects.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of the various cases of absence of the pulmonary artery involves chiefly their differentiation from other cases in which there is only a single arterial trunk. Their differentiation from cases of atresia depends on whether or not an atretic remnant of the pulmonary artery is present, and a careful dissection must be made to rule out this possibility. Atresia of the aorta with the pulmonary artery remaining as the solitary arterial trunk presents what is sometimes a confusing picture, but if carefully studied it is not likely to be mistaken for absence of the pulmonary artery. The only difficulty arises in distinguishing absence of the pulmonary artery from persistent truncus arteriosus communis, largely because cases of absence of the pulmonary artery have been previously presented under the latter diagnosis. Once the concept of absence of the pulmonary artery is appreciated, there is little difficulty in differentiating the two defects.

There is some disagreement in the present literature as to the correct diagnosis of persistent truncus arteriosus communis. Abbott^{7a} in 1931 classified 23 cases as instances of this anomaly in her review of 1,000 cases of congenital heart disease, but Humphreys⁹ in 1932 accepted only 12 cases and added another of her own. In 1942 Lev and Saphir¹⁰ collected 12 additional cases, and since then 5 others have been reported by Doerr,¹¹ Van Brown,¹² Marshall,¹³ Hunter⁴ and Webb,¹⁴ making a total of 30 cases according to the present standards of diagnosis. On the other hand, Shapiro¹⁵ in 1930 found only 2 anomalies described in the literature that he would accept as true examples of persistent truncus arteriosus communis. Humphrey's

9 Humphreys, E. M. Arch Path **14** 671, 1932

10 Lev, M., and Saphir, O. J Pediat **20** 74, 1942

11 Doerr, W. Virchows Arch f path Anat **310** 304, 1943

12 Van Brown, D. J Tech Methods **22** 101, 1942

13 Marshall, R. Brit Heart J **5** 194, 1943

14 Webb, A. C. Arch Path **42** 427, 1946

15 Shapiro, P. F. Arch Path **10** 671, 1930

report contained a thorough review of the subject, and her work has received wide acceptance. She attempted to clarify the situation by establishing definite diagnostic criteria. The principal criteria which she evolved were the presence of a single large arterial trunk which shows the combined features of the aorta and the pulmonary artery, the trunk preferably showing four semilunar valve cusps. Secondary features noted were dextroposition of the trunk, in the "rider" position above an interventricular septal defect, and certain modifications of the crista supraventricularis and of the mitral and tricuspid leaflets. She described as "partial" types (partial defect of the aortopulmonary septum) those in which there is a main pulmonary trunk arising from the ascending aorta and dividing into right and left pulmonary branches, and as "complete" types those in which the pulmonary trunk is missing and right and left pulmonary arteries arise independently from the aorta. Included among the "complete" types were those in which these vessels arise from the arch or from the descending aorta.

As previously discussed, we believed that cases of the latter types in which the lungs are supplied by the ductus arteriosus (from the arch) or by bronchial arteries (from the descending aorta) should be classified as cases of absence of the pulmonary artery. The absence of the sixth aortic arch is the fundamental and primary defect in these instances, occurring before the fourth week of embryonal development. Without the development of the sixth arch the stimulus or the nidus for development of the aortopulmonary septum (fourth and fifth weeks) is lacking. Therefore, the designation "absence of the pulmonary artery" indicates the fundamental defect which is responsible for the final structure of the heart, while the use of the term "persistent truncus arteriosus communis" merely describes the secondary defect, the failure of development of the aortopulmonary septum. It may be argued that the single trunk does represent persistence of the undivided primitive truncus arteriosus. This may be true, but in the final anomaly which we see at the autopsy table, this single trunk represents anatomically nothing more than the aorta. It can hardly be considered a common trunk of the aorta and the pulmonary artery when the pulmonary artery does not exist. Therefore, we suggest that an additional essential criterion for the diagnosis of persistent truncus arteriosus communis is that the pulmonary artery or arteries arise from the ascending aorta proximal to the point of origin of the innominate artery.

There is an interesting and challenging question concerning the number of semilunar cusps present in the valves of the single arterial trunks. It is admitted that with failure of the aortopulmonary septum to divide the common truncus arteriosus, its four distal bulbar swellings

should give rise to a valve containing four semilunar cusps. Consequently all cases of persistent truncus arteriosus communis should show four cusps, but in less than half of the reported cases has this been true. An attempted explanation of this apparent paradox is that an abnormal number of semilunar cusps is occasionally found in even normal hearts and that the cases of common arterial trunk displaying only three valve cusps could represent a coincidental variant. However, this explanation seems wanting, especially when it is observed that the incidence of an abnormal number of valve cusps among all types of congenital heart disease is less than 5 per cent^{7a}, yet the incidence of three-cusped valves in the reported cases of persistent truncus arteriosus communis is greater than 50 per cent. This is an interesting question, and one that if answered might shed considerable light on the development of congenital cardiac anomalies. It is interesting to note that of the cases of single arterial trunks showing four valve cusps reported, all but 1 belong to the type that we have designated group 1 (persistent truncus arteriosus communis), and none have been found in our group 3 (absence of the pulmonary artery with hypertrophy of the bronchial arteries).

In addition to the embryologic basis for separating the cases of absence of the pulmonary artery from those of persistent truncus arteriosus communis, there is an interesting, if somewhat surprising, clinical correlation between the two. In the 29 cases of persistent truncus arteriosus communis the average age of survival was less than two months (excluding two of Humphrey's "possible cases" in which the patients lived 16 and 25 years), whereas in the 7 cases of absence of the pulmonary artery with hypertrophied bronchial arteries the average age of survival was more than eleven years. Thus, persistent truncus arteriosus is more likely to arouse interest at the autopsy table, while absence of the pulmonary artery may assume more clinical importance.

ABSENCE OF THE PULMONARY ARTERY WITH HYPERTROPHIED BRONCHIAL ARTERIES

Our case is an example of absence of the pulmonary artery with compensatory hypertrophy and dilatation of the bronchial arteries. A review of the literature reveals that 6 similar cases have been reported (although under different diagnoses), 1 each by Hulse,⁶ Zimmerman,¹⁶ Finley,¹⁷ Miller and Lyon,¹⁸ Greenspon and Leaman¹⁹ and Solis-

16 Zimmerman, H. M. *Am J Path* **3** 617, 1927.

17 Finley, K. H. *Am J Path* **6** 317, 1930.

18 Miller, M. K., and Lyon, M. W., Jr. *Am Heart J* **7** 106, 1931.

19 Greenspon, S., and Leaman, W. G., Jr. *Internat Clin* **4** 208, 1939.

Cohen and associates²⁰ The latter 2 cases were reported as instances of complete atresia and atresia of the pulmonary artery, respectively, although no remnant of the pulmonary artery was present If no trace of the artery is seen, the term "atresia" is incorrect, for atresia means closure of a normal opening or channel rather than absence of a structure Furthermore, the term "complete atresia" is redundant, for atresia is actually complete stenosis The other cases were reported as instances of persistent truncus arteriosus Such a diagnosis would identify the single trunk as the common stem of the aorta and the pulmonary artery when actually there is no pulmonary artery It would indicate that the fundamental embryonal fault was a failure of development of the aorticopulmonary septum, but, as already mentioned, this could not produce the lesion in question, and furthermore it hardly seems logical to describe a failure of a septum between two vessels when one of those vessels does not exist

If the fundamental defect in these cases is considered to be an absence or an early involution of the primitive sixth aortic arch, then the complete picture can be readily explained With the absence of the sixth arch, the pulmonary artery fails to be established and the pulmonary plexus maintains the parts by which it communicates with the ventral branches of the dorsal aorta, which become the bronchial arteries The subsequent increase in the volume of blood flowing through these vessels produces compensatory hypertrophy and dilatation, which enable them to supply sufficient blood for aeration of the lungs At the base of the heart the abnormal flow of blood streaming from both ventricles into the aorta prevents the closing of the basal or membranous portion of the interventricular septum and is also probably responsible for the incomplete rotation or detorsion that leads to the partial dextroposition of the aorta so that the latter is in the "rider" position The dilatation of the root of the aorta naturally follows its increased quantity of blood, and the hypertrophy of the ventricles is a result of the cardiac embarrassment produced by the abnormal circulation Thus these cases may be classified as absence of the pulmonary artery with compensatory dilatation and hypertrophy of the bronchial arteries, this designation giving both an accurate anatomic description and a clear indication of the underlying embryonal process leading to the development of the anomaly

It is amazing how efficiently the bronchial arteries are able to take over in the absence of the pulmonary artery As stated before, the average age of the 7 patients in this group was over 11 years, 3 of them reaching adulthood This efficient bronchial substitution

20 Solis-Cohen, M, Zaslow, J, and Rolnick, M H Am Heart J 28 115, 1944

is also seen to a striking degree in cases of atresia of the pulmonary artery, which functionally are similar to cases of absence of the pulmonary arteries Christeller²¹ reported 5 cases in which the patients lived from 16 to 37 years Bach²² recorded 1 case in which the age of 30 was attained, and East and Barnard²³ cited 2 cases in which the patients reached the ages of 20 and 33, and neither of these patients died of their cardiac anomalies

There are two mechanisms whereby this compensatory function of the bronchial arteries may be derived, dependent on the stage of development at which the lesion of the pulmonary artery occurs In the cases of atresia, in which the pulmonary artery has once been established, the bronchial arteries probably take over by the process of increasing their anastomotic channels within the lung Although ordinarily no anastomosis is considered to exist between the pulmonary and bronchial circulations, Miller²⁴ has demonstrated by a series of intravascular injections that an indirect anastomosis is present through their common venous capillary plexus That this is capable of developing into a free communication is evidenced by the cases cited On the other hand, in cases of primary failure of the sixth arch, in which the pulmonary artery has never formed, it is probable that the independently developing pulmonary plexus maintains its connection with the ventral branches of the dorsal aorta, as mentioned before, and in this way the bronchial arteries are developed from the very beginning into efficient pulmonary vessels

CLINICAL DIAGNOSIS

Although it is perhaps not possible to determine clinically the exact type of anomaly that exists in these cases, it may be possible to ascertain that one is dealing with either absence or atresia of the pulmonary artery with abnormal blood supply to the lungs The general clinical picture may be similar to the well known tetralogy of Fallot except that there may be no murmurs One may expect the usual stigmas of congenital heart disease, such as cyanosis, clubbing of the fingers and toes and polycythemia, but these signs are often not as marked as in the tetralogy, for the hypertrophied bronchial arteries function more efficiently than do the stenosed pulmonary arteries It is also well to keep in mind that some of these patients may not present any symptoms of cardiac disease for a number of years The electrocardiogram should show a right ventricular pre-

21 Christeller, E Virchows Arch f path Anat **223** 40, 1917

22 Bach, F Lancet **1** 1009, 1928

23 East, T, and Barnard, W G Lancet **1** 834, 1938

24 Miller, W S The Lung, Springfield, Ill, Charles C Thomas, Publisher, 1937

ponderance, and the roentgenogram will usually show the typical "coeur en sabot" outline of the heart. Danelius²⁵ reported a case in which a review of the roentgenograms after autopsy showed absence of the hilar shadow, and he expressed the belief that if this had been noticed and its significance appreciated, a correct antemortem diagnosis could have been made. He reviewed previously reported cases and found the same sign in many of them, suggesting that absence of the hilar shadow may be of some diagnostic importance in cases of absence or atresia of the pulmonary artery with anomalous pulmonary blood supply. However, our case failed to show this sign. Apparently it may be of positive value if present, but of no negative value if not present.

Thus, if these anomalies are kept in mind, a presumptive clinical diagnosis may be made in many of these cases. Knowledge of this would of course be of particular value to the surgeon contemplating intrathoracic correction of a defect, so that he might be able to avoid damaging any abnormal vessels encountered which might later prove to be the only ones supplying blood to the lungs or to one or more lobes of the lungs. At least two such unfortunate accidents that resulted in operative deaths have been reported²⁶

SUMMARY

Congenital cardiac anomalies involving a single aortic trunk with an abnormal site of origin of the pulmonary artery are discussed on the basis of their embryonal development and with particular reference to their recognition.

A case of absence of the pulmonary artery in which the lungs were supplied solely by hypertrophied bronchial arteries is presented. A review of the literature reveals that 6 other cases of this type have been reported. However, they were reported under various other diagnostic headings because of the fact that there had never been an adequate classification of these cases.

A new classification for this group of cases is presented which places emphasis on the maldevelopment of the pulmonary artery as the fundamental lesion in producing the final anatomic defect. It is felt that this suggested classification will help to clarify the interpretation and diagnosis of these cases.

Congenital cardiac anomalies in which there is a single arterial trunk and an abnormal site of origin of the arteries supplying the lungs may be satisfactorily classified as follows:

1. Persistent truncus arteriosus communis (incomplete development of the aorticopulmonary septum)

25 Danelius, G. *Am J Roentgenol* 47:870, 1942

26 Blount and Poth^{7c}; Harris and Lewis^{7e}

- 2 Absence of the pulmonary artery with persistent ductus arteriosus (absence of the ventral root of the sixth arch with persistence of the left dorsal root)
- 3 Absence of the pulmonary artery with hypertrophied bronchial arteries (absence of entire sixth arch)
- 4 Absence of the pulmonary arteries with anomalous pulmonary vessels (absence of entire sixth arch)

Atresia of the pulmonary artery is similar to absence of the artery except for the mechanism of its formation, and the classification presented may be equally well applied to cases of this condition

It is suggested that an additional requirement for the diagnosis of persistent truncus arteriosus communis is that the pulmonary arteries arise from the common trunk proximal to the origin of the innominate artery

The value of adequate recognition of these cases is discussed with respect not only to their further study but also to the application of modern methods of surgical treatment

RADIORESISTANT CELLS IN CERTAIN RADIOSENSITIVE TISSUES OF SWINE EXPOSED TO ATOMIC BOMB RADIATION

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THAT the threshold of tolerance to total body-penetrating ionizing radiation varies among the several species of plant and animal life is well known. In addition to species variations in radiosensitivity there are individual variations, usually of smaller magnitude, among members of the same species. However, it is interesting that in mammals as a class specific tissues bear a fairly constant radiosensitivity relationship to one another.

Irradiation of the whole body affords an effective means for study of the radiosensitivity differences of the various tissues of a living animal. However, two important filtration factors must be considered when one is calculating the dose of total body irradiation that any particular tissue receives. The lack of uniform density in the body tissues introduces the greatest single variable, but also of importance is the thickness of the animal which, owing to the shielding effect of interposed organs, may lead to errors in the calculation of the dose delivered to distal tissues. Because of the amount and the penetrating quality of the radiations, the use of atomic energy as the source of total body irradiation tends to minimize, but probably does not entirely eliminate, the effect of differences due to specific density of the tissue and body thickness of the organism.

One advantage in studying swine is that one is able to reproduce the differences in tissue density and body thickness variables which are present in man. In addition, swine are believed to be in roughly the same sensitivity range as man. For these reasons, among others, they were chosen as test animals for the Bikini atomic bomb trials in 1946¹.

The principle of total body irradiation is a concept not generally familiar to the medical profession, but is one which takes on added significance as man advances in the atomic age. Judicious therapeutic doses of ionizing radiation localized on a tumor destroy the tumor

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1. Draeger, R. H., and Warren, S. U. S. Nav. M. Bull. **47** 219, 1947. Tullis, J. L., and Warren, S. J. A. M. A. **134** 1155, 1947.

without marked or lasting effect on the host in general. Yet it is known that with 1,000 kilovolt roentgen rays, at 1 meter distance from the source 600 roentgens (r), measured in air delivered at the rate of 30 r per minute to both lateral aspects, or something more than 1,200 r measured in air delivered to one lateral aspect, is the absolute lethal dose for swine². Although such comparatively small doses delivered in a short period have a lethal effect on the swine, many cells and organs show no morphologic evidence of injury. The radioresistant tissues are usually highly differentiated and include nerve, muscle, bone and cartilage.

The tissues of the Bikini swine which showed the greatest morphologic changes were the lymphoid organs—including lymph nodes, the spleen, the thymus, the tonsils and lymphoid aggregations in the bowel wall, as well as lymphocytes in the peripheral blood stream—the bone marrow, the intestinal epithelium and the gonads³. The comments in this report are limited to observations concerning the morphologic changes noted in these radiosensitive tissues.

LYMPHOID CELLS AND TISSUES

Lymphocytes are probably the most radiosensitive cells in the body. Following irradiation there is a prompt reduction in circulating lymphocytes and in the lymphocyte population of the lymphoid organs. The lymphocytes undergo rapid lysis in some cases, but in others they undergo a more leisurely degeneration and can be spotted as free or as phagocytosed nuclear debris. In lymph nodules destruction of lymphocytes serves to demonstrate by contrast the survival of the reticular cells (fig 1). Recent observations on animals exposed to external and internal ionizing radiations from atomic, radioactive isotopes and roentgen ray sources have established the fact that reticular cells are relatively radioresistant as judged by morphologic standards⁴.

However, in lymph nodes and the spleen the macrophages are not altered structurally, and functionally they appear to be hyperactive. Erythrophagocytosis in these tissues occurs with greater than normal frequency. This should not imply that stimulation of the macrophages has occurred, but simply that they are keeping abreast of their increased work load, namely, the phagocytosis of injured red blood cells, degenerating white blood cells and blood pigment. Evidence of erythrophagocytosis is found in most of the spleen and lymph nodes examined of those animals which died the second day or later after exposure (fig 2).

² Tullis, J. L., Tessmer, C. F., Cronkite, E. P., and Chambers, F. W., Jr. *Radiology* 52: 396, 1949.

³ Tullis, J. L. *Am J Path*, to be published.

⁴ (a) Bloom, W. *Histopathology of Irradiation from External and Internal Sources*, New York, McGraw-Hill Book Company, Inc., 1948. (b) Tullis³.

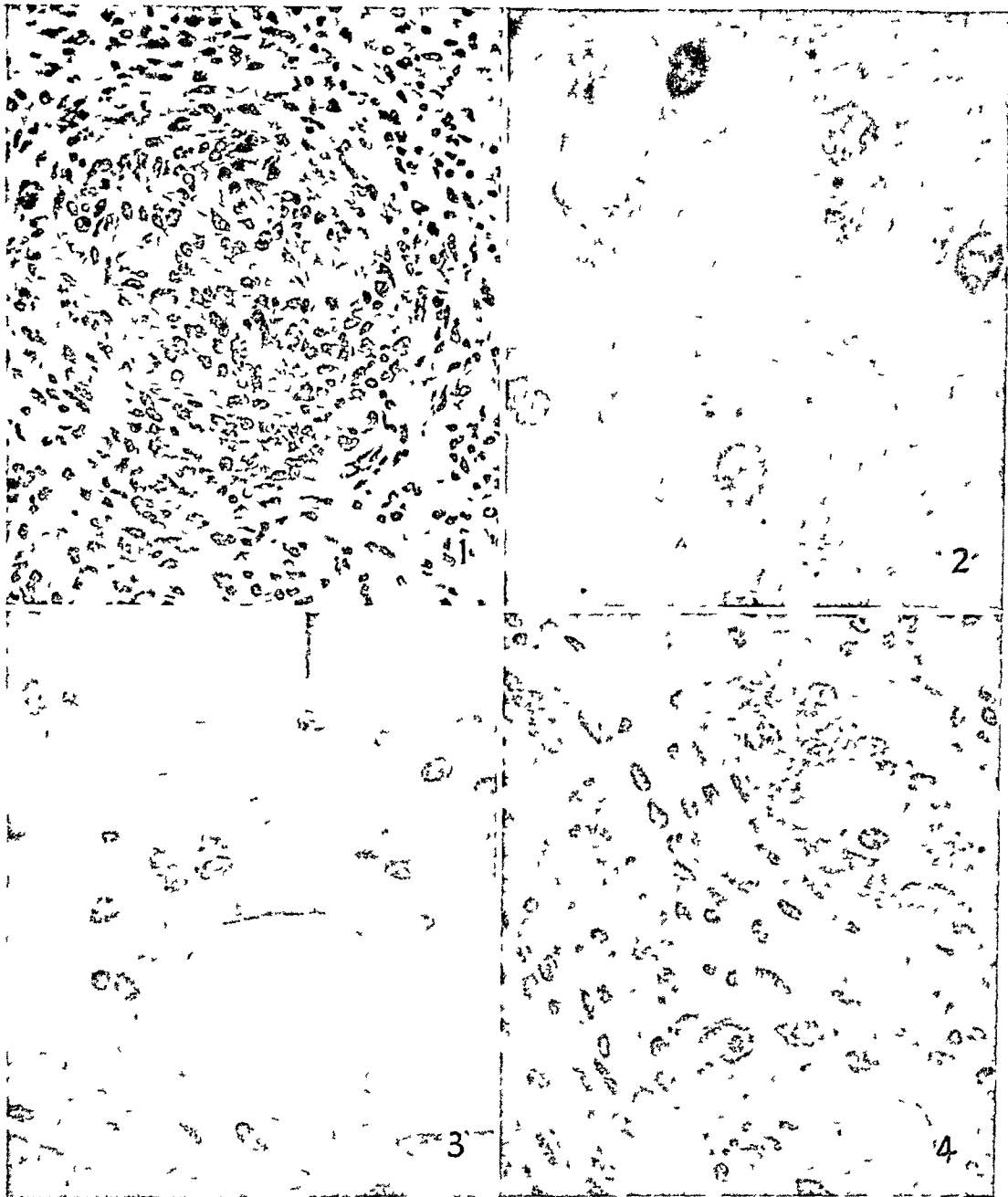


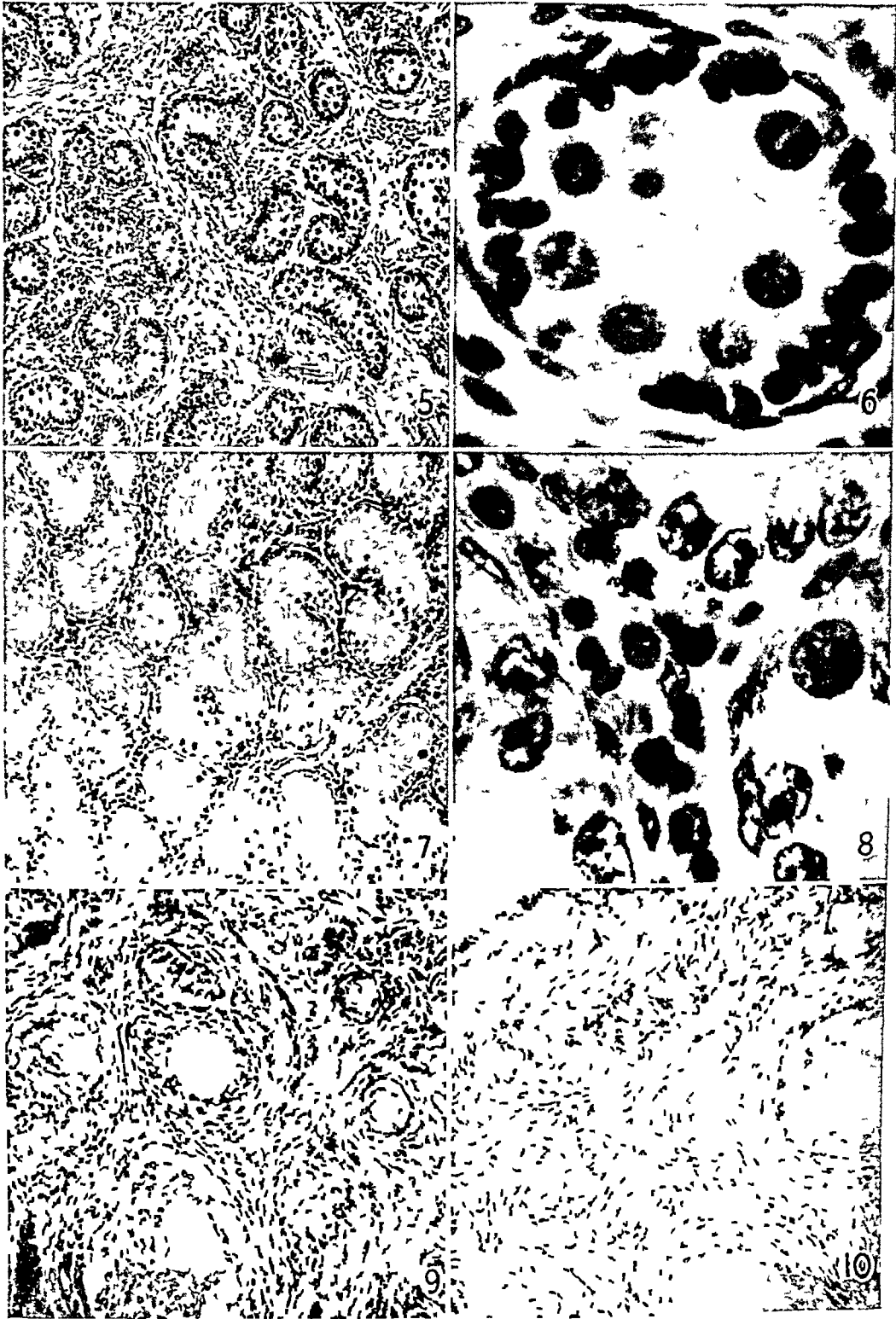
Fig 1—Lymph node from pig 265 six days after irradiation. Most of the lymphocytes in this nodule have been destroyed leaving numerous, apparently normal, reticular cells exposed to view $\times 300$

The swine mentioned in this and subsequent legends received over 1,000 rem total body irradiation during Test Baker (underwater explosion) at Bikini. The abbreviation rem stands for the roentgen-equivalent man-unit of energy absorbed in tissue biologically equivalent in man to 1 r of roentgen or gamma radiation.

Fig 2—Lymph node from pig 393 eleven days after irradiation. Two phagocytes containing red blood cells and blood pigment granules are seen $\times 1,350$

Fig 3—Bone marrow from pig 265 six days after irradiation. Reticular cells in an amorphous homogeneous matrix, fat cells and erythrocytes outnumber other cellular elements $\times 645$

Fig 4—Small intestine from pig 419 five days after irradiation. The cells lining the glands show marked variation in size, shape and distribution of chromatin. Many of the cells in the lamina propria are pyknotic, while others have completely disappeared $\times 400$



FIGURES 5-10
(See legends on opposite page)

BONE MARROW

In the bone marrow the blast cells of both the red and the white cell series are very radiosensitive while the more mature cells are less radiosensitive. However, even after doses of total body irradiation sufficient to destroy nearly all the maturing cells in the marrow, the reticular cells remain intact (fig 3). Investigators are thus faced with an interesting phenomenon in which blast cells and maturing and mature cells are destroyed, leaving intact only the elementary stem cells, as has been described by others⁵

EPITHELIUM OF BOWEL

The intestinal epithelium is radiosensitive and reacts in a variety of ways. Complete destruction of the epithelium with ulceration of the muscularis mucosae may occur at random, chiefly in the large and less frequently in the small bowel. The reason for the localization of the ulcers is not apparent, although the presence of lymphoid aggregations in the basal lamina propria may possibly be a precipitating factor. There may be no remnants of lymphoid elements, and no unusual feature may be observed which would account for the localization of the ulcers. The margins of the necrotic tissue are sharply demarcated, often with one row of partially damaged glands forming the boundary between ulcerated and uninvolved areas. The cells of the marginal glands and glands elsewhere with minimal involvement show considerable variation in size, shape, amount and distribution.

5 Shouse, S. S., Warren, S. L., and Whipple, G. H. *J. Exper. Med.* **53**: 421, 1931. Bloom, M. A., and Bloom, W. *J. Lab. & Clin. Med.* **32**: 653, 1947.

EXPLANATION OF FIGS 5-10

Fig 5—Testis from control pig 250. Numerous spermatogonia are observed in the tubules. Spermatocytes and lumens have not yet formed in the tubules. $\times 150$

Fig 6—Testis from control pig 250. The distribution and relative numbers of spermatogonia and indifferent cells are seen. $\times 300$

The swine mentioned in these legends, except pig 250 (control), received over 1,000 rem total body irradiation during Test Baker (underwater explosion) at Bikini.

Fig 7—Testis from pig 330 thirteen days after irradiation. Most spermatogonia and all but a few spermatocytes have been destroyed. The larger size of the tubules, the beginning of the formation of lumens and the presence of spermatocytes indicate that pig 330 was two to three weeks older than pig 265. Compare with figure 4. $\times 150$

Fig 8—Testis from pig 330. One spermatogonium is seen in this portion of three tubules, indicating extensive destruction of these cells. The indifferent cells are unharmed. The amorphous material in the central portion of the tubules is vacuolated. $\times 300$

Fig 9—Ovary from pig 364 seven days after irradiation. The ova and the surrounding follicular cells are vacuolated. $\times 150$

Fig 10—Ovary from pig 402 thirteen days after irradiation. The primordial ova and the ovarian stroma appear normal. $\times 150$

of chromatin in the nuclei (fig 4) The epithelium in neighboring glands may have large vacuolated nuclei or pyknotic nuclei Again there is no apparent local determining factor to account for the type of reaction observed

It is noteworthy that the lymphocytes in the lamina propria are greatly diminished, but that the fibrocytes and plasmacytes are apparently uninjured

GONADS

The same pattern of response observed in the lymphoid organs and the bone marrow is also manifest in the testis The Bikini swine were 3 to 3½ months of age at the time of the experiment, and the males had just begun to mature sexually

On histologic examination it is observed that the lumens of the tubules are beginning to form Many stem or so-called "indifferent" cells, a few spermatogonia and, in some areas, a few spermatocytes are present (figs 5 and 6) No fully formed sperm is observed Within five days after lethal total body irradiation most of the spermatogonia and spermatocytes disappear The cytoplasm of the tubular cells and the amorphous interstitial material of the tubules become granular, stringy and vacuolated (fig 7) On the other hand, the indifferent cells which line the tubules next to the basement membrane show no structural alterations, nor are they reduced in number (fig 8)

In the ovary the primordial ova seem to be more resistant to irradiation than the developing ova The latter show such changes as vacuolation of the cytoplasm of the follicular cells and lysis and fragmentation of the chromatin of the ova (fig 9) In most cases in swine the primordial ova show no morphologic changes (fig 10), but this evidently does not hold true for all species^{4a} The ovarian stroma is radioresistant but may become slightly edematous after total body irradiation

COMMENT

The significance of these observations is concerned with the fact that the reticular cells of the lymphoid organs and the bone marrow, the indifferent cells of the immature testis and the primordial ova, all of which cells are the so-called stem cells of their respective highly radiosensitive organs, are comparatively radioresistant This poses an important exception to the law of Bergonie and Tribondeau⁶ (primitive cells are more sensitive to radiation than specialized cells), which should be modified to mention specifically that the stem cells are excluded in the progression of radiosensitivity postulated

6 Bergonie, J, and Tribondeau, L Compt rend Acad d sc **143** 983, 1906

The qualities which make such cells as the reticular cells of lymphoid organs and bone marrow, fixed and circulating macrophages, and plasmacytes relatively much more radioresistant than the closely related lymphocytes are not apparent morphologically

The purpose of this paper is to point out that there are certain radioresistant elements in the more radiosensitive organs of the body with the hope that this knowledge will cause the physiologist and the histochemist to focus their search for the mechanism of ionizing radiation injury on the metabolic and chemical differences of the radiosensitive as compared with the radioresistant elements. The great importance attached to the radioresistance of certain stem cells is concerned with recovery of the organism from radiation injury. It is apparent that if treatment during the acute phase of the injury is successful in maintaining life, there is histologic evidence that regeneration of radiosensitive cells can be accomplished by the surviving stem cells.

EFFECT OF TRIPELENNAMINE HYDROCHLORIDE ON ACUTE INFLAMMATION

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ALTHOUGH many workers¹ have attributed the sequence of events in acute inflammation to the influence of chemical substances which are released from injured tissue, such substances have not been precisely identified. In particular, much conflicting evidence exists regarding the role of histamine or an "H substance" in the acute inflammatory process.²

The demonstration³ that such antagonists of histamine as diphenhydramine (benadryl® [beta dimethylaminoethyl benzhydryl ether]), tripeleennamine (pyribenzamine® [N,N-dimethyl-N'-benzyl-N'-(alpha-pyridyl) ethylenediamine]) and pyranisamine (neo-antergan® [N,N-dimethyl-N'-(p-methoxybenzyl)-N'-(alpha-pyridyl) ethylenediamine]) markedly alter the response of the skin to histamine suggested their use to investigate the part played by histamine in acute inflammation.

It was postulated that if histamine caused the local vasodilatation and increased permeability of capillaries noted in inflammation, as was suggested by Lewis,⁴ the administration of an antagonist of histamine such as tripeleennamine would alter the process of acute aseptic inflammation.

From the Department of Pathology, Northwestern University Medical School
1 Ebbecke, U. *Arch f d ges Physiol* **169** 1, 1917. Lewis, T, and Grant, R. T. *Heart* **11** 209, 1924. Dale, H. H. *Lancet* **1** 1233, 1929. Menkin, V. *Dynamics of Inflammation. An Inquiry into the Mechanism of Infectious Processes*, New York, The Macmillan Company, 1940.

2 (a) Menkin, V. *J Exper Med* **64** 485, 1936, (b) *Proc Soc Exper Biol & Med* **40** 103, 1939, (c) *J Exper Med* **67** 129, 1938. (d) Bier, O, and Pocha e Silva, M. *Arq Inst biol São Paulo* **9** 109, 1938, (e) *Compt rend Soc de Med* **129** 769, 1938. (f) Rous, P, and Gelding, H. P. *J Exper Med* **51** 27, 1930. (g) Wayne, E. J. *Heart* **16** 53, 1931. (h) Grant, R. T, and Jones, T. D. *ibid* **14** 336, 1927.

3 Arbesman, C. E., Koepf, G. F, and Miller, G. F. *J Allergy* **17** 203, 1946. Yonkman, F. F., Chess, D., Mathieson, D., and Hanson, N. *J Pharmacol & Exper Therap* **87** 122, 1945. Friedlander, S., and Feinberg, S. *J Allergy* **17** 129, 1946.

4 Lewis, T. *The Blood Vessels of the Human Skin and Their Responses*, London, Shaw & Sons, 1927.

MATERIALS AND METHODS

Effect of Vasodilatation—Two groups of female albino rabbits were used. In series A, consisting of 10 rabbits, three areas of acute inflammation were induced on the ventral aspect of the ear by ten seconds' application of a metal rod, 0.9 cm in diameter, which had been heated to 90°C. In series B, consisting of 11 rabbits, three intradermal injections of 0.02 cc of turpentine served as the irritant. Since it was noted that the area of erythema was maximal three minutes after application of the stimulus, the erythematous areas were transilluminated, traced and measured with a planimeter at this time. The average of three measurements was used.

Shortly after the control measurements had been taken, each animal was given intravenously 40 mg of tripeleminamine hydrochloride⁵ per kilogram of body weight. Thirty minutes later the same stimuli were applied to analogous areas on the opposite ear, and the resulting erythema was measured.

Histamine skin wheals were produced in 20 rabbits by an intradermal injection of 0.02 cc of a 1:20,000 solution of histamine phosphate in sterile isotonic solution of sodium chloride. The injections were made in areas corresponding to those used for the procedures described in the foregoing paragraphs, before and after administration of tripeleminamine hydrochloride. The resulting erythema was measured.

Effect of Permeability of Capillaries—Inflammatory responses to burns, turpentine and histamine were produced, as previously described, on the shaved abdomens of 12 rabbits. Seven of these animals had received intravenously 40 mg of tripeleminamine hydrochloride per kilogram of body weight thirty minutes before the stimuli were applied, and 5 served as controls. The permeability of capillaries was estimated by the intensity of the concentration of dye three, thirty and one hundred and eighty minutes after 10 mg of trypan blue had been injected into the marginal vein of an ear.

Rabbits 1 through 9 were normal rabbits. Rabbits 10 through 20 had been used earlier for the Friedman test, but the abdominal wounds were healed and the animals were in good health. In experiments requiring repeated injections of tripeleminamine hydrochloride, at least forty-eight hours were allowed to elapse between injections. No anesthesia was used.

Microscopic sections of tissues fixed in formaldehyde solution and stained by the hematoxylin-eosin technic were made at varying intervals after the inflammatory stimulus had been applied.

EFFECT ON VASODILATATION

Burns (series A)—After the administration of tripeleminamine, the areas of erythema were reduced in all animals. The reductions ranged from 25 to 78 per cent as compared with the control measurements of the burns on the opposite ear. The average reduction was 43 per cent (table 1).

Injections of Turpentine (series B)—Reduction of the erythema was noted in 7 of the 10 animals after the administration of tripeleminamine. The decreases ranged from 11 to 62 per cent as compared with

⁵ Pyribenzamine hydrochloride,[®] supplied by Ciba Pharmaceutical Products, Inc., was used.

the control measurements The average reduction was 29 per cent (table 2)

Histamine Wheals (series A and B) —Tripelennamine reduced the erythema from 19 to 85 per cent as compared with the control measurements The average reduction was 47 per cent (table 3)

No correlation was noted between the reduction of the histamine flare and that of the erythema following each of the other stimuli.

TABLE 1—*Areas of Erythema Following Standard Burns in Square Millimeters*

Rabbit	Before Tripelennamine	After Tripelennamine	Reduction, Per Cent
1	92	70	23
2	79	28	65
4	131	103	21
5	94	71	25
6	123	93	25
7	156	73	54
11*	132	72	46
12*	125	27	78
13*	91	57	38
14*	101	65	35
			Av reduction 43 per cent

* This rabbit was previously used for the Friedman test

TABLE 2—*Areas of Erythema Following Injections of Turpentine in Square Millimeters*

Rabbit	Before Tripelennamine	After Tripelennamine	Reduction, Per Cent
5	129	90	31
6	108	96	11
7	108	106	0
8	192	107	44
10*	245	223	9
15*	318	148	53
16*	290	167	42
17*	211	158	25
18*	319	121	62
19*	356	346	3
20*	204	93	55
			Av reduction 29 per cent

* This rabbit was previously used for the Friedman test

These measurements were made on different days The intensity of the erythema, as well as the area, was decreased in all animals

EFFECT ON PERMEABILITY OF CAPILLARIES

In the studies of permeability of capillaries, no reduction of the intensity of dye concentration was noted following the administration of tripelennamine hydrochloride when burns and injections of turpentine were used as the stimuli, but the localization following injections of histamine phosphate was completely prevented (table 4)

6 Footnote deleted by the authors

No alteration of cellular exudate was noted microscopically. It is difficult to judge vasodilatation from tissue sections.

The doses of tripeleennamine hydrochloride used produced increased irritability and restlessness in the animals and, if given rapidly, caused convulsions. Although the area of erythema following a standard stimu-

TABLE 3—*Areas of Erythema Following Injections of Histamine Phosphate in Square Millimeters*

Rabbit	Before	After	Reduction, Per Cent
	Tripeleennamine	Tripeleennamine	
1	122	18	85
2	57	10	82
3	95	67	32
4	119	97	19
5	154	35	78
6	104	70	30
7	106	84	20
8	146	47	76
9	163	112	31
10*	131	84	36
11*	130	46	68
12*	145	62	51
13*	97	60	39
14*	88	51	43
15*	122	78	35
16*	159	33	78
17*	118	65	46
18*	137	81	41
19*	170	78	53
20*	188	35	85
			Av. reduction 47 per cent

* This rabbit was previously used for the Friedman test.

TABLE 4—*Intensity of Trypan Blue Concentration at Sites of Inflammation*

Rabbit	Administration of Tripeleennamine	Concentration		
		Histamine	Burns	Turpentine
1	Yes	Negative	3 plus	
4	Yes	Negative	3 plus	1 plus
6	Yes	Negative	2 plus	Negative
7	No	1 plus	3 plus	2 plus
8	Yes	Negative	2 plus	2 plus
10*	No	2 plus	2 plus	1 plus
11*	Yes	Negative	2 plus	
14*	Yes	Negative	2 plus	
15*	No	2 plus	2 plus	2 plus
21*	No	2 plus	3 plus	

* This rabbit was previously used in the Friedman test.

lus varied from animal to animal and from day to day in the same animal, it was remarkably constant in the same animal on the same day. No difference of response was noted between the normal rabbits and those used previously for Friedman tests.

COMMENT

In these studies tripeleennamine was effective in reducing the vasodilatation that followed the intradermal injection of concentrations of

histamine phosphate comparable to those occurring in inflammatory exudates. Tripeleennamine's antagonism to histamine is thought to resemble that described by Wells and associates⁷ for diphenhydramine (benadryl®), which is a competitive adsorption of the drug on the effector cells sensitive to histamine. Since the drug shows no atropine-like effects, it is considered to be more specific as an antagonist of histamine than diphenhydramine⁸.

Interpretation of the reduction of the erythema produced by a burn, as well as of that following the injection of a foreign substance such as turpentine, following the administration of tripeleennamine requires further study. This effect might be due to stimulation of any of the vasoconstrictor mechanisms or to inhibition of the vasodilator mechanism, as well as to an action on some substance produced locally by trauma.

The failure of tripeleennamine to alter the intensity of the concentration of trypan blue following burns and injections of turpentine, while completely obliterating that following injections of histamine phosphate, suggests that substances other than histamine are responsible for the increased permeability of capillaries involved in inflammation. Menkin^{2a} has attributed this increased permeability to leukotaxine, and Collumbine⁹ has shown recently that the action of leukotaxine is not altered by pyranisamine (neo-antergan®), an antagonist of histamine.

SUMMARY

In these experiments tripeleennamine reduced the erythema and the increase in permeability of capillaries that follow the intradermal injection of concentrations of histamine comparable to those found in inflammatory exudates. The drug reduced the erythema that followed burns and injections of turpentine under similar experimental conditions, but failed to alter the increase of capillary permeability that follows the application of these irritants. No alteration of the microscopic picture of acute inflammation was noted after administration of the drug.

⁷ Wells, J. A., Morris, H. C., Bull, H. B., and Dragstedt, C. A. *J. Pharmacol. & Exper. Therap.* **85** 122, 1945.

⁸ Sherrod, T. R., Loew, E. R., and Schloenner, H. P. *J. Pharmacol. & Exper. Therap.* **89** 247, 1947.

⁹ Collumbine, H. *Nature, London*, **159** 841, 1947.

GLIOMA OF THE NOSE

Report of a Case of the Extranasal Type

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ALTHOUGH congenital glioma of the nose is clinically benign, it has considerable interest because of its rarity and because of the unusual location. In most of the reported cases there is a subcutaneous tumor forming an external swelling in the region of the bridge of the nose (extranasal type)¹. In a few cases the tumor has been wholly within the nasal cavity (intranasal type)². In other cases the tumor has been both extranasal and intranasal (mixed type)³. Glioma of the nose must be differentiated from gliomatous encephalocele (brain hernia), which occurs in the same locations but which has a definite intracranial connection that can be traced through a defect in the bone beneath the tumor.

REPORT OF CASE

A white infant, a boy weighing 2,500 Gm, was delivered from a primipara 20 years of age after an uneventful pregnancy and labor (Dr Margaret Clark, of Lawrence, Kan, gave me permission to use the case). He appeared normal except for a tumor on the left side of the bridge of the nose, which presented no unusual widening. The tumor, which was covered by intact skin, appeared to be a dome-shaped mass measuring about 12 mm in diameter across its broad base. There had been no unusual maternal illnesses during the pregnancy. At the end of six days the tumor had not definitely increased in size but was removed for cosmetic reasons by sharp dissection, with the infant under local anesthesia. The base of the wound, which lay almost on the periosteum, was fulgurated since it was believed that all of the tumor tissue had not been removed. The preoperative impression was that the tumor was a hemangioma. There had been no recurrence eight months later, and the scar was almost unnoticeable.

The specimen measured 16 mm in diameter and 6 mm in thickness. It was dome shaped, and over the surface there was intact epidermis. This rather soft

From the Department of Pathology, University of Kansas School of Medicine

1 (a) Schmidt, M B. *Virchows Arch f path Anat* **162** 340, 1900 (b) Sussenguth, L. *ibid* **195** 537, 1909 (c) Cominos, O. *Arch d'opt* **31** 177, 1911 (d) Berblinger, W. *Centralbl f allg Path u path Anat* **31** 201, 1920 (e) Davis, E W. *J Neuropath & Exper Neurol* **1** 312, 1942 (f) Bratton, A B, and Robinson, S H G. *J Path & Bact* **58** 643, 1946

2 (a) Clark, J P. *Am J M Sc* **129** 769, 1905 (b) Schwartz, A H, and Isaacs, H J. *Arch Otolaryng* **34** 838, 1941

3 (a) Rocher, H L, and Anglade. *Rev de chir* **62** 147, 1924 (b) Clark^{2a}

specimen cut without increased resistance, and the cut surface had a light grayish, somewhat gelatinous appearance with a small central light brown area of softening

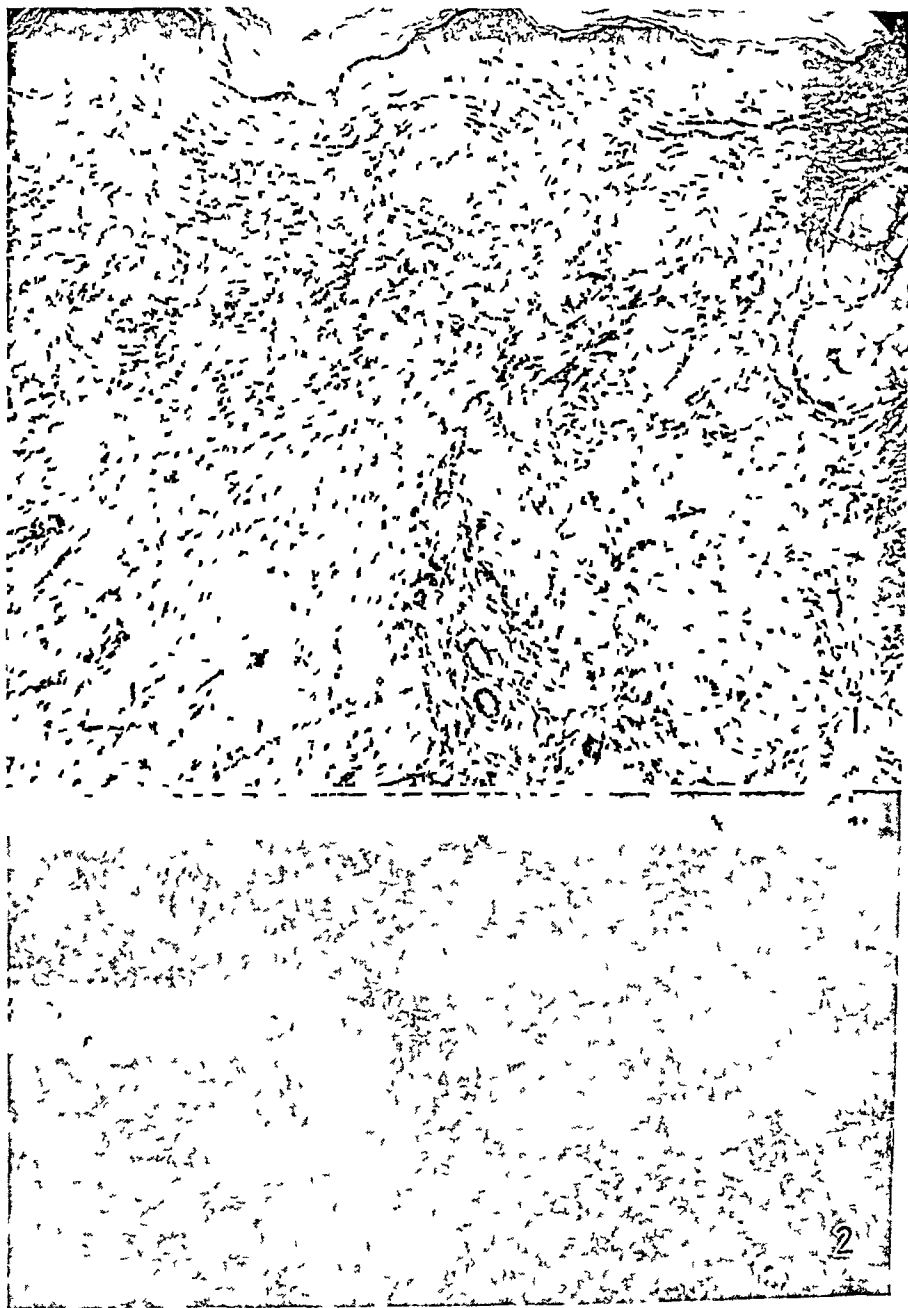


Fig 1—Photomicrograph of tumor showing masses of glial tissue beneath intact epidermis, phosphotungstic acid-hematoxylin, $\times 70$

Fig 2—Photomicrograph of tumor showing atypical astrocytes, Cajal's gold chloride-mercuric chloride mixture, $\times 400$

Histologic examination revealed in the corium a large mass of delicate fibrillar tissue extending to the margin of excision (fig 1). There were varying numbers of capillaries and a few foci of hemorrhage. The nuclei had a fairly

uniform appearance, although a few were large and somewhat vesicular. In hematoxylin-eosin preparations the cytoplasm was indistinct except for the few multinucleated cells, which had a moderate amount of light pink cytoplasm. In sections stained with phosphotungstic acid-hematoxylin and with Cajal's gold chloride-mercuric chloride mixture, there were numerous branching cells, some of which were rather large and had coarse, elongated processes (fig 2). In some fields these glia-like fibers were loosely arranged, while in others they formed compact masses, usually with a parallel arrangement.

Diagnosis—Glioma of the nose, extranasal type

COMMENT

Encephalocele (brain hernia) may occur in the same locations as nasal glioma and, moreover, may form a solid tumor with a gliomatous aspect.⁴ In some of the cases the intracranial connection is narrow and may be merely a fibrous cord. In reports of 2 cases of nasal glioma, mention is made of defects in the bone beneath the tumor which were sealed over with fibrous tissue,^{1c, e} so that prior existence of an intracranial connection might have been suspected. In view of these facts it is reasonable to suppose that the nasal glioma is derived from an encephalocele of embryonal life in which the part connecting it with the brain is lost through closure of the frontal bones.

Doubt has been expressed as to the neoplastic character of the glial tissue (Guthrie and Dott⁵, Browder^{4b}), the inference being that the atypical astrocytes represent hyperplasia or gliosis rather than a true neoplasm. The histologic aspect is similar to that of a well differentiated astrocytoma, and the growth characteristics, i. e., failure to recur or to metastasize, do not afford a distinction between glioma and gliosis. In some cases of nasal glioma and gliomatous encephalocele the tumor has increased in size but not in others. In some cases the tumor has been removed before enough time has elapsed for growth to be detected. Certainly, the factors which Penfield⁶ stated to be favorable to gliosis (circulatory impairment, death of cerebral tissue and obliteration of the subarachnoid and perivascular spaces) must exist in cases of encephalocele. Other observers⁷ have compared the astrocytic tissue in cases of glioma of the nose with the cerebral glioses. Davis applied the term "nasal fibroglial heterotopia" to the finding in his case. It is surprising

4 (a) Fevre, M., and Huguenin, R. *Ann d'anat path* **13** 333, 1936. (b) Browder, J. *Ann Otol, Rhin & Laryng* **38** 395, 1929. (c) Rawson, J. D., and Vivoli, D. *Rev med latino-am* **14** 860, 1929. (d) Rocher and Anglade^{3a}

5 Guthrie, D., and Dott, N. *J Laryng & Otol* **42** 733, 1927

6 Penfield, W. *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 457

7 Comminos^{1c} Rocher and Anglade^{3a}

to discover that only well differentiated glial tumors have been observed. At the present time there is insufficient evidence to abolish the use of the term "glioma of the nose."

Accurate differentiation between glioma and encephalocele of the nose requires detailed anatomic study either by adequate surgical exposure or by necropsy to determine the presence or the absence of an intracranial connection. This problem, which is well presented by Zollner,⁸ has practical importance, since in some cases encephalocele has cerebrospinal fluid channels, and death has resulted from meningitis following removal of a gliomatous encephalocele.

A clinicopathologic comparison of gliomas and neuroblastic tumors of the nasal fossa has been presented by Portmann and Beillard,⁹ and a similar comparison has been made by Jemmi.¹⁰

New and Devine¹¹ referred to 3 cases in which a small encephalocele appeared to be similar if not identical with glioma of the nose, but a detailed description is not presented. Reference has been made to a possible case of nasal glioma by Eggston,¹² but again a detailed description is lacking.

In my case fibrous tissue did not form an integral part of the proliferative process, while in other instances it has formed a prominent tissue element leading to the term "fibroglioma." The fibrous tissue was considered homologous with the pia mater by Schmidt. In many cases gemistocytic astrocytes and Nissl's plump cells have been described. They were not numerous in my case.

Pathologically, nasal glioma is to be differentiated from encephalocele, meningoencephalocele, ganglioneuroma, neurinoma, neurofibroma and a nerve tumor of the olfactory tract. Clinically, angioma, lipoma and dermoid cyst have to be considered.

SUMMARY

A rare congenital benign glial tumor occurring at the root of the nose is described which probably arose from ectopic brain tissue separated by an anomaly of development in early embryonic life. Although the neoplastic character of the growth is debatable, retention of the term "glioma of the nose" seems desirable.

8 Zollner, F. Frankfurt Ztschr f Path **49** 82, 1935

9 Portmann, G and Beillard, P. F. Rev de laryng, otol, rhin **68** 125, 1947

10 Jemmi, C. Oto-rino-laring ital **15** 149, 1947

11 New, G. B., and Devine, K. D. Arch Otolaryng **46** 163, 1947

12 Eggston, A. A., and Wolff, D. Histopathology of the Ear, Nose and Throat, Baltimore, Williams & Wilkins Company, 1947, p 774

Case Reports

CARCINOID OF THE RECTUM

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CARCINOID originating in the rectum appears to be quite rare, since only 14 cases were found described in the available literature. Furthermore, in reviewing the characteristics of the tumor in these cases, it was discovered that in only 2 cases did it have an affinity for the silver stain. In addition, metastases were noted in 2 other cases.

Since a recently observed example of carcinoid of the rectum showed both argentaffinity and a metastasis, it was considered of sufficient interest to report.

R I W, a 57 year old white man, entered the hospital with signs and symptoms suggestive of a recurrence of a ruptured intervertebral disk that had been treated surgically four years previously. On physical examination a 3 by 5 cm firm, hard tumor, resting on a broad base, was discovered 5 cm within the rectum on the left wall. There was a history of five bouts of mild and painless rectal bleeding six months before.

It was considered that the tumor was of primary concern, and conservative treatment of the back pain produced satisfactory results during the subsequent investigations. Roentgen studies of the chest and the gastrointestinal tract revealed no abnormalities. The tumor was not demonstrated in the roentgenograms. Serologic tests gave negative results, and the blood and urine were normal.

Microscopic examination of a specimen removed from the edge of the tumor showed rectal mucosa abruptly terminating in dense fibrous tissue infiltrated by nests of small round cells of fairly uniform character with solid, dark nuclei. Further specimens were requested. A second specimen consisted of twelve bits of tissue. Sections revealed normal mucosa in which there were some areas of glandular hyperplasia with piling-up of the cells into several layers. The basement membranes, as seen, appeared intact. In the area of the tunica propria there were diffuse to densely packed islands of cells as described. The muscularis could be seen to be invaded by these cells.

A one stage abdominoperineal resection was performed. The pathologic report of the surgical specimens follows.

"The specimens consist of an anorectal piece of bowel, 20 cm long, and a 36 cm length of sigmoid bowel.

"Lower piece. 2 cm from the anorectal junction there is a round, firm, pseudo-lobulated, yellow tumor 2.6 cm in diameter. It rests on a base 2 cm in diameter. The mucosa runs up to the side of the tumor and stops. On cut section the

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tumor is firm, yellow and composed of various-sized lobules. It is dense in consistency and attached to the submucosal tissues. No infiltration is detected grossly. At the upper end of the piece of bowel is an elongated hard lymph node, 3.5 by 1 cm., within the fatty tissue. On section this node is morphologically identical with the tumor. The upper piece of bowel reveals no abnormality.

"Microscopic sections (hematoxylin and eosin) reveal portions of the surface to be covered with an atrophic layer of rectal mucous membrane. At one point, however, there are changes to hyperplastic proliferation. The submucosa is heavily infiltrated with plasmacytes, lymphocytes, eosinophils and tumor cells. In the submucosa and muscularis there is a marked desmoplastic reaction with a heavy interlacing network of fibrous connective tissue containing tumor cells. These



Argentaffin carcinoid of the rectum

cells are growing in cords, and form sheets and columns in their stroma. The cells have medium to abundant cytoplasm containing occasional fine granules and many vacuoles of varying sizes. The nuclei are of varying sizes and shapes but are generally large and irregularly oval in outline. They are irregularly granular but are usually deeply so. Mitotic figures are present in small numbers.

"With Masson's silver stain, the bulk of the tumor cells do not accept the silver, and it is observed that the cytoplasm of these cells is not granular. Argentaffin cells occur in clumps and are of larger than average size. The argentaffin granules are fine and are usually unipolar, but may be bipolar, and are occasionally diffusely distributed throughout the cytoplasm."

Early convalescence of the patient was uneventful, with exercises started the fourth day. It was difficult to keep him out of bed. On the twenty-second postoperative day, after taking a bath, the patient was seized with painless dyspnea,

followed by cyanosis, collapse and death within a few minutes. Autopsy revealed massive emboli in both pulmonary arteries. The pelvic region had a dry, subsiding fibrinous peritonitis with some retroperitoneal adenopathy and a number of thrombosed small veins. No metastases were found.

COMMENT

Several different theories have been advanced for the origin of carcinoid of the intestine. The recent literature appears generally in agreement that the cells of origin are within the depths of the crypts of Lieberkuhn.¹ The argentaffinity of the Kultschitzky cells, as well as their chemical, morphologic and developmental similarities to carcinoid cells are provocative.²

Argentaffin basigranular cells occur throughout the gastrointestinal tract. Argentaffin carcinoids have been reported³ occurring in all portions of the intestinal tract. The percentage of these tumors accepting the silver stain is much higher in the small bowel and appendix than in the rectum.

Eispaimer and others¹ have postulated "pre-enterochrome" and "enterochrome" states of the basigranular cells, that the staining reaction, or presence of, granules depends on the metabolic state of the cell at the moment of fixation. Either confirmation or disproof of this theory would make it appear of little profit to separate these poorly understood tumors into two classes because of the silver staining properties of a fraction of the cells in some of them.

SUMMARY

A case of an argentaffin carcinoid of the rectum is reported

1 Cited by Stout, A. P. *Am J Path* **18** 993, 1942

2 Masson, P. *Am J Path* **4** 181, 1928

3 Porter, J. E., and Whelan, C. S. *Am J Cancer*, **36** 343, 1939

Laboratory Methods and Technical Notes

GIEMSA STAIN

AT THE April 16 meeting of the Biological Stain Commission, the Board of Trustees voted that hereafter Giemsa stain will be certified in two varieties, namely, "Giemsa stain, azure B type, for malaria and blood work" and "Giemsa stain azure A type for hematology and bacteriology."

The azure B type closely resembles tinctorially and spectroscopically the Grubler and Hellborn Giemsa stains of the 1930's and is the variety especially recommended to give the faintly greenish blue tint to parasite cytoplasm which contrasts well with the grayish or greenish blue background of the thick film stained at p_H 7.0. This is recommended by many malariologists for thick film work.

The azure A type gives darker red chromatin stains, grayer or more violet blue lymphocyte cytoplasm and perhaps somewhat heavier staining of micro-organisms. Its useful life is probably shorter under average tropical storage conditions. It is preferred by many American hematologists.

Notes and News

Appointments, Retirements, Etc—Appointment of Dr H G Grady as scientific director of the American Registry of Pathology has been announced by Brig Gen Raymond O Dart, director of the Army Institute of Pathology. Dr Grady succeeds Col James E Ash, United States Army Medical Corps (Ret), who is retiring from active participation in registries.

Eleonius T Bell, professor of pathology at the University of Minnesota Medical School, Minneapolis, retired June 15. He has served the university since 1910, teaching first anatomy and since 1911 pathology. He was appointed head of the department in 1921. An advisory committee has recommended the establishment of a fund of \$100,000 to create and maintain for teaching and research a museum of pathology in the medical school, which will bear his name. James S McCartney Jr is chairman of the E T Bell Fund Committee.

Richard H Shryock, Ph D, professor of American history and since 1938 lecturer in medical history at the University of Pennsylvania, Philadelphia, has been appointed director of the Institute of the History of Medicine and William H Welch professor of the history of medicine at the Johns Hopkins University School of Medicine, Baltimore. The new director will assume his duties in September. The Institute of the History of Medicine was founded by Dr William H Welch in 1926. Dr Welch, the first director, was followed by Dr Henry Sigerist. Dr Shryock became interested in medical history while serving with the Medical Corps during World War I. Returning to the University of Pennsylvania after the war, he continued training for the degree of Doctor of Philosophy.

Prizes, Awards, Etc—According to the *Journal of the American Medical Association* a David Anderson-Berry silver-gilt medal, together with a sum of money amounting to about 100 pounds, will be awarded in 1950 by the Royal Society of Edinburgh to the person who in the opinion of the Council has recently produced the best work concerning the therapeutic effect of roentgen rays on human diseases. Applications for this prize are invited. They may be based on both published and unpublished work and should be accompanied by copies of relevant papers. Applications must be in the hands of the General Secretary, Royal Society of Edinburgh, 22 George Street, Edinburgh 2, by March 31, 1950.

Fellowships—The American Cancer Society, in conjunction with the British Empire Cancer Campaign, announces a number of British American Exchange Fellowships in Cancer Research. These fellowships are open to American citizens who possess the degree of M D, Ph D or D Sc and will provide specialized training in Great Britain, an equal number of young British scientists will be selected for training in this country. The fellowships, to be awarded by the society on recommendation of the Committee on Growth of the National Research Council, will be granted for a period of one year at \$4,020, with travel allowance of \$600. Applications may be procured from the Executive Secretary of the Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D C.

Society News—The 1950 meetings of the American Association of Pathologists and Bacteriologists will be held at the University of Wisconsin, Madison, April 14 and 15, 1950.

The nineteenth annual meeting of the Biological Photographic Association, Inc, will be held in Cleveland on Wednesday, Sept 7, 1949 through Saturday noon Sept 10, 1949 at the Hotel Cleveland.

Books Received

Oral Anatomy By Harry Sicher, M.D., professor of anatomy and histology, Loyola University School of Dentistry (Chicago College of Dental Surgery) Pp 529, with 310 illustrations (24 in color) Price, \$15 St Louis C V Mosby Company, 1949

This book is dedicated to the memory of Julius Tandler (1869-1936), formerly professor of anatomy at the University of Vienna, Austria, and shows as its frontispiece a medallion portrait of the head of that eminent teacher. As is stated in the preface, the book is based on a German text written by Sicher in collaboration with Tandler (Sicher and Tandler *Anatomie für Zahnärzte*, Berlin, Julius Springer, 1928). Though much of the book represents a translation of that text, and about 250 of the 310 figures are either from it or from Tandler's "*Lehrbuch der systematischen Anatomie*" (Tandler *Lehrbuch der systematischen Anatomie*, Leipzig, F. C. W. Vogel, 1923-1929, vol. 4), there are some added sections, especially those dealing with the more recent observations on the growth of the skull and that of the alveoli in relation to the teeth, and there are about 35 new illustrations. The remainder of the figures were taken from previous publications by various authors. There is a failure to give parenthetically in the explanation of the figures either the titles of the bibliographic references or their place and date of publication.

As Sicher observes rightly, one of the most difficult problems of teaching anatomy or other basic sciences is that of correlation. Largely because of the arrangement of the curriculum, most students in a dental school—and in some medical schools—do not become aware of the applicability of theory to practice. When the student takes his course in anatomy he cannot apply his knowledge because he has not yet had an insight into the clinical problems in which anatomic knowledge is indispensable, and when later he starts his clinical training he cannot apply his anatomic knowledge because he has forgotten most of it. Since time is short, a review of anatomic details in a course of applied anatomy is then almost impossible, consequently "clinical teaching and learning are more and more mechanized and the student arrives at the conclusion that he can become a successful dentist without much knowledge in basic sciences." Sicher's book tries to bridge the gap between theory and practice. It endeavors not only to replace selected chapters of a textbook of anatomy in the freshman course but also to accompany the student through his clinical years by serving as a basic introduction to some of the practical courses. The book is intended to supplement textbooks on human anatomy, which are written primarily for use in medical schools, and the chapters of which dealing with the head and neck are at the same time too broad and not deep or special enough for the dentist or the oral surgeon.

The subject matter of the book is arranged in two parts: the first, *descriptive anatomy* of the regional divisions, and the second, *regional and applied anatomy*, in which the principle of arrangement is the clinic. The seven chapters of the first part deal respectively with (1) the skull, (2) the muscles of the head and neck, (3) the temporomandibular articulation, (4) the viscera, (5) the blood vessels, (6) the lymphatic system and (7) the nerves of the head and neck. The eight chapters of the second part are designated as follows: (1) palpability of the facial skeleton, (2) structure and relations of the alveolar processes, (3) anatomy of local anesthesia, (4) arterial hemorrhages and ligation of arteries, (5) the propa-

gation of dental infections, (6) tracheotomy and laryngotomy, (7) the temporo-mandibular articulation and (8) the edentulous mouth. Though the treatment of the subject matter is chiefly gross-anatomic, there is in chapter 1 an excellent section on the growth of the skull, including facial growth in relation to tooth eruption. The gross histologic details of the relations of the teeth to the tissues of their sockets also are well shown. The functional anatomy of the temporo-mandibular articulation and the muscles of mastication is considered adequately, but not so that of the other parts.

Since one of the merits of the book is the large number of illustrations, it is unfortunate that the figures, which were so excellent in the German text, so greatly lost their crispness and distinctiveness during the process of reproduction in this American text. It is generally a mistake to photograph half-tone figures and to prepare new half-tone etchings from them instead of using the original clichés (which in this case obviously was impossible), the contrasts between the highlights and the shadows are flattened. A grave error which the author and the publisher could have avoided, if they had been alert, is the different reductions during the reproduction of the figures. It is disconcerting when the various views of the skull and the different bones of the same skull are not shown in a constant size relation to each other. This oversight, the reviewer believes, has definitely impaired the quality of the book. Another criticism which may be leveled at many morphologists is their failure to indicate either the enlargement or the diminution of the actual view of the structure shown where an indication of the size relations is important for comparison. For none of the photomicrographs, for example, has Sicher expressed the magnifications employed.

The text is presented in a straightforward and matter-of-fact style. What it may lack in fluency, it gains in clarity, precision and simplicity of expression, which must be the dominant aim of the morphologist in his descriptions.

All in all, the reviewer regards Dr. Sicher's "Oral Anatomy" as the best book of its kind in English, and he congratulates both the author and the publisher for their achievement.

THE CHEMISTRY AND TECHNOLOGY OF ENZYMES. By Henry Tauber, Ph.D. Pp. 550, with 56 illustrations. Price, \$7.50. New York: John Wiley & Sons (London: Chapman & Hall), 1949.

This book is an expansion of an earlier work, "Enzyme Technology," by the same author, and, as the title implies, it is divided into two sections: one on the general chemistry of enzymes and the second on the applications of enzymology to technology. The first section can be recommended as a useful guide to the chemistry of enzymes for the experimental pathologist. In addition to a generally orthodox classification of known enzymes and discussion of their properties it includes reasonably complete instructions for determination of a few of the more commonly measured and more easily measurable enzymes. This section will serve its most useful purpose as a guide to the literature since it is extensively documented. Its usefulness could perhaps have been extended by the inclusion of a more comprehensive section on enzyme kinetics and the fundamental considerations involved in devising a quantitative assay procedure for measuring enzyme activity. Present day enzyme research is striving for the recognition of the action of individual enzymes in organized reaction systems. There is little discussion in the book of enzyme systems such as those involved in glycolysis, urea formation, etc. Although such discussion more properly is a matter of the

biochemistry of intermediary metabolism, perhaps a chapter devoted to outlining a few systems would give a more dynamic conception of the action of enzymes.

The reviewer does not feel competent to judge the section on enzyme technology, a subject which is perhaps of less interest to the experimental pathologist. It will suffice to list a few of the subjects covered: Industrial production of yeast, ethyl alcohol by fermentation, brewing, production of antibiotics, baking processes, dairy technology, food preservation and tanning. Some material on microbiologic assay of certain vitamins and amino acids is included and should prove useful as orientation.

On the whole, the reviewer feels that this book, considering only the section on chemistry of enzymes, is not as useful a general text as "Chemistry and Methods of Enzymes," by Sumner and Somers, for the nonchemist research worker in the medical sciences. This opinion does not reflect on Dr. Tauber's effort, since he obviously has written the book for a different audience.

QUANTITATIVE STUDY OF THE EFFECTS OF MULTIPLE SUCCESSIVE CLOSED CEREBRAL LESIONS

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AND
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PREVIOUS quantitative studies of acute closed cerebral lesions produced by a hypothermal method showed a good correlation between symptoms and volumes of cerebral damage per hundred volumes of the brain in rabbits¹. The average maximum quantity of damage permitting survival of 50 per cent of the animals was 14.3 volumes per hundred volumes of the brain. No animal survived with cerebral damage in excess of 18.5 per cent of the brain. Most animals with cerebral damage of these magnitudes recovered promptly from the anesthesia after production of lesions and then, after several hours of normal behavior, lapsed into an unconscious state which terminated in death. It seemed that factors which led to the delayed onset of symptoms were also restricting the amount of acute closed cerebral injury which could be produced without a fatal result. The purpose of the present study was to investigate these factors so that therapy of acute and subacute progressive cerebral damage might be analyzed in a quantitative way by experimental methods.

METHODS

Albino rabbits, 3 to 6 months of age and weighing 4 to 6 pounds (about 2 to 3 Kg), were used.

The closed intracerebral hypothermal lesions were produced by a technic described elsewhere¹. Briefly, this consisted of aseptic exposure of the calvarium at the vertex of the skull of an anesthetized animal and controlled freezing of the calvarium and underlying cerebrum with a special instrument. Lesion 1 was made in the left occipitoparietal region, care being taken to produce a sublethal lesion. The scalp was then closed with silk sutures. If the animal survived, the procedure was repeated after the lapse of a chosen interval of time. A second lesion (lesion 2) of sublethal volume was then produced in the right occipito-

This study was aided by a grant from the Otho S. A. Sprague Memorial Fund. From the Rush Department of Pathology, Presbyterian Hospital, in affiliation with the Department of Pathology, University of Illinois College of Medicine.

1 Taylor, C. B., Hass, G. M., and Maloney, J. E. Relations Between Volumes of Closed Hypothermal Cerebral Lesions and Symptoms in Rabbits, Arch. Path. 47:450, 1949.

parietal region Lesions 3, 4, 5 and 6 were then produced successively at the same intervals of time in the left parietal, right parietal, left frontal and right frontal regions, respectively In most instances, animals failed to survive the complete series of lesions, so that the number of lesions varied from two to six

There were four groups of animals Successive cerebral lesions were produced at intervals of twenty-four hours in 12 rabbits, forty-eight hours in 12 rabbits, seventy-two hours in 4 rabbits and ninety-six hours in 4 rabbits Repeated clinical observations were made, and a record was kept of the time of onset and the duration of paralysis, stupor, coma or convulsions, as well as the duration of life after production of the last lesion Five types of clinical courses based on the variable responses of the animals were recognized

If an animal lived for twenty-four hours after the production of a lesion, it was regarded as a survivor in accordance with previous experience¹ Each experiment, therefore was terminated either when the animal died of lethal lesions or was killed twenty-four hours after production of the final lesion

The determination of the volumes of cerebral damage per hundred volumes of the brain was made by methods described elsewhere¹ These involved measurement of the volume of the brain and the volume of each lesion The ratio between the sum of the volumes of individual lesions and the total volume of the brain was taken as a standard approximation of the percentage volume of cerebral damage

RESULTS

The characteristics of acute closed cerebral lesions produced by the hypothermal method have been described previously¹ They were discrete circumscribed lesions characterized principally by necrosis, hemorrhage and edema of the cerebral cortex and underlying white matter The necrosis affected all cerebral elements within the sharp boundaries of each lesion and was uniform in nature There was no suppuration, and structure of connective tissues was well preserved Hemorrhage was restricted to the region of injury Free bleeding into the subarachnoid space was not encountered Bleeding into the ventricles occurred only when lesions involved ventricular walls, but this complication was not encountered in the present study, because lesions of great depth were not produced Edema was grossly apparent in the lesions and adjacent cerebral tissue

The production of successive lesions spaced at intervals of several hours led to three complications which made evaluation of data more difficult than in the experiments previously reported Reopening of surgical wounds of the scalp led to infection at times, but this did not seem to contribute to death of animals The second complication was overlapping of adjacent lesions at their margins Because of this, errors in the calculation of percentage volume of cerebral injury were greater than when lesions were separated from one another by normal brain The third complication was the gradual decrease in size of lesions as they healed This introduced an error in the determination of percentage volume of cerebral injury The error was always in underestimating the actual percentage volume of damage

Previous experience indicated that rabbits could tolerate less than 94 volumes of acute cerebral damage per hundred volumes of brain without symptoms When the amount of cerebral damage was 94 to 185 per cent of the brain, symptoms, at times leading to death, occurred in many animals (see chart 1) The minimum volume that would lead to death of 50 per cent of the animals (MLV₅₀) was 143 per cent of the brain All animals with acute closed cerebral injury in excess of 185 per cent died

For these reasons, it was necessary to control the volume of acute injury at each successive stage of the experiment. Each acute lesion had to be small, so that the summation of effects of successive lesions would not be unduly obscured by the effect of the most recent acute lesion. The data in table 4 show the maximum, minimum and average percentage volumes of cerebral injury, as represented by single lesions. The maximum size of any single lesion in the series was 13.4 per cent of the brain. The average size of lesions was about 4 to 6 per cent. There was little difference among the dimensions of lesions in the four groups of animals with spacing of lesions at twenty-four, forty-eight, seventy-two and ninety-six hour intervals. It was concluded that the slight variability in size of lesions had little influence on differences in behavior of the four groups of animals.

The limits of survivable damage in the four groups of animals are shown in tables 1, 2 and 3. (See also chart 2.) When lesions were produced at intervals of twenty-four hours, the maximum survivable damage was 22.6 per cent. This was produced by three successive lesions. Attempts to exceed this value resulted in death of the animals. The remaining 11 animals in this group behaved in the same manner as animals with comparable injury produced by multiple simultaneous

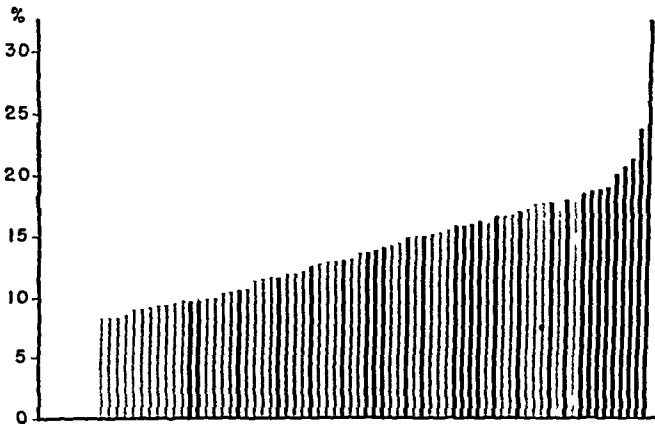


Chart 1—Each bar represents an animal with acute unilateral or bilateral cerebral lesions produced at one operation. The height of the bar indicates the percentage volume of cerebral damage. Each interrupted bar represents an animal which survived and each solid bar an animal which died.

lesions¹. Therefore, the spacing of successive lesions at intervals of twenty-four hours had about the same effect as if the lesions had all been produced at one time.

The production of successive cerebral lesions at intervals of forty-eight hours led to survival of 5 of 12 animals with 20.2 to 29.1 volumes of cerebral injury per hundred volumes of brain. This was a definite indication that successive lesions had less effect when the interval between lesions was forty-eight rather than twenty-four hours.

Three of 4 animals with lesions produced at intervals of seventy-two hours survived with 22.4, 23.6 and 25.9 volumes of cerebral injury, respectively, per hundred volumes of brain. Similarly, 4 of 4 animals with lesions produced at intervals of ninety-six hours survived with 20.9, 26.8, 27.5 and 29.2 volumes of cerebral injury, respectively, per hundred volumes of brain. These data indicate that although there was some advantage to the animal if lesions were produced at intervals greater than forty-eight hours, the advantage was not great in terms of either survival or recuperation of cerebral function.

The signs and symptoms which the animals showed after the production of each successive lesion were recorded. Five types of clinical courses were recognized. Type 1 was characterized by rapid recovery from the operation without subsequent symptoms, type 2, by a delayed recovery of consciousness in the immediate postoperative period without subsequent symptoms, type 3, by a normal or slightly delayed recovery of consciousness in the immediate postoperative period, followed by an asymptomatic return to normal behavior, then a lapse into stupor and coma with death before the twenty-fifth postoperative hour, type 4, by a lack of recovery of consciousness following the operation, with death occurring usually within two to four hours, type 5, by a persistent stupor or vegetative state, which usually continued for days without much change. The animals assumed a semi-reclining posture, moved about sluggishly on stimulation and did not eat well. No well defined motor paralysis was encountered in any of the five types of clinical courses.

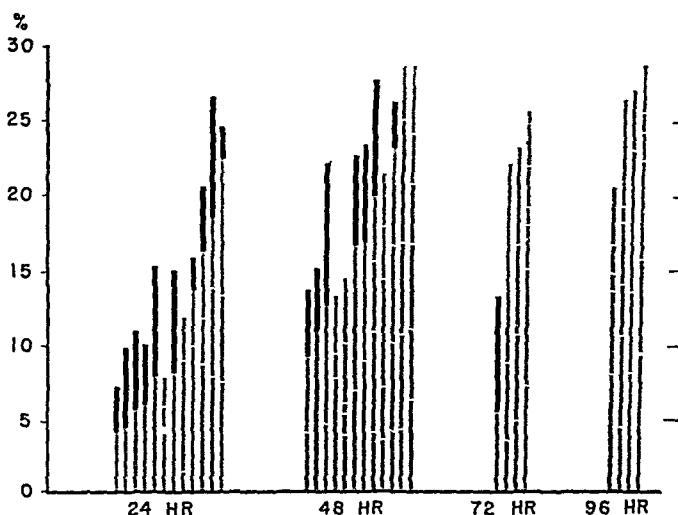


Chart 2—The total height of each bar indicates the sum of successive percentage volumes of cerebral damage in an animal. Each bar is subdivided into columns which represent the individual volumes of each successive lesion. The interval of time between operations producing successive lesions is indicated at the base of each group of bars. When the final lesion resulted in death, the percentage volume of the brain occupied by the final lesion is indicated by the length of the terminal solid section of the bar.

Previous experience with acute unilateral or acute simultaneous bilateral lesions showed that there was good correlation between the type of clinical course and the percentage volume of cerebral damage. The type of clinical course was clearly established within twenty-four hours after the operation. No new signs or symptoms developed, thereafter, except in the animals which lost weight and strength during the prolonged stupor of the type 5 clinical course. In the acute experiments involving a single operation it was found that when the quantity of cerebral damage was less than 14.3 per cent of the brain, most animals followed clinical courses of type 1 and type 2. When the quantity of cerebral damage was about 14.3 per cent of the brain, most animals followed clinical courses of types 3, 4 and, in rare instances, 5. No animal survived when the cerebral damage was greater than 18.5 per cent of the brain.

The present experiments dealing with successive acute cerebral lesions produced at intervals of twenty-four hours gave the following relations between the percentage volume of cerebral damage and the type of clinical course (See also table 1)

Type 1 range of volume of cerebral damage 13 to 137 per cent, average, 72 per cent

Type 2 range, 42 to 186 per cent, average, 106 per cent

Type 3 range, 99 to 152 per cent, average, 124 per cent

Type 4 range, 71 to 267 per cent, average, 191 per cent

Type 5 range, 118 to 226 per cent, average, 153 per cent

A comparison of these data and those reported previously shows that multiple lesions spaced at intervals of twenty-four hours led to essentially the same result

TABLE 1—*Clinical Results of Increasing the Percentage Volume of Cerebral Damage by Producing Successive Cerebral Lesions at Intervals of Twenty-Four Hours**

Experiment Number	Percentage Volume of Brain Lesion 1 †	Percentage Volume of Brain Lesions 1 and 2	Percentage Volume of Brain Lesions 1 to 3	Percentage Volume of Brain Lesions 1 to 4	Percentage Volume of Brain Lesions 1 to 5
83	68 (1)	137 (1)	226 (3)	251 (4)	
1	80 (1)	141 (2)	186 (2)	267 (4)	
84	89 (1)	121 (5)	164 (5)	208 (4)	
10	102 (1)	138 (5)	160 (4)		
14	91 (1)	118 (5)			
16	13 (1)	81 (1)	152 (3)		
15	42 (1)	46 (1)	59 (1)	79 (1)	81 (1)
8	79 (1)	155 (3)			
85	60 (1)	101 (3)			
86	57 (2)	111 (3)			
17	44 (1)	99 (3)			
6	42 (2)	71 (4)			

* The types of clinical courses are indicated by numerals 1 to 5 in parentheses adjacent to the percentage volume of cerebral damage

† In this and subsequent tables the percentage volume represents the ratio between the total volume of the lesions and the volume of the brain

as similar lesions produced simultaneously, with the exception that multiple spaced lesions were more frequently followed by a type 5 clinical course. These animals always had a large total volume of cerebral damage in proportion to volume of brain

When acute cerebral lesions were produced successively at intervals of forty-eight hours, the following correlations between type of clinical course and percentage volume of cerebral damage were obtained (See table 2)

Type 1 range, 38 to 172 per cent, average, 90 per cent

Type 2 range, 110 to 255 per cent, average, 159 per cent

Type 3 range, 139 to 290 per cent, average, 219 per cent

Type 4 range, 230 to 282 per cent, average, 256 per cent

Type 5 range, 147 to 291 per cent, average, 218 per cent

These data show that the quantity of cerebral damage could, on the average, be increased about 40 to 50 per cent in representative surviving groups, such as type 2 and type 5, if lesions were spaced at intervals of forty-eight rather than

twenty-four hours. It is to be noted that as the volume of cerebral damage was progressively increased the animals tended to follow successively the clinical courses in the order type 1, type 2, type 5.

When multiple cerebral lesions were produced successively at intervals of seventy-two and ninety-six hours, the following correlations between clinical course and percentage volume of cerebral damage were obtained (See also table 3)

TABLE 2—*Clinical Results of Increasing the Percentage Volume of Cerebral Damage by Producing Successive Cerebral Lesions at Intervals of Forty-Eight Hours**

Experiment Number	Percentage Volume of Brain Lesion 1	Percentage Volume of Brain Lesions 1 and 2	Percentage Volume of Brain Lesions 1 to 3	Percentage Volume of Brain Lesions 1 to 4	Percentage Volume of Brain Lesions 1 to 5	Percentage Volume of Brain Lesions 1 to 6
42	6.4 (1)	11.3 (2)	17.0 (1)	24.8 (5)	29.1 (5)	
43	4.5 (1)	11.0 (2)	17.3 (2)	25.5 (2)	29.0 (3)	
62	4.3 (1)	10.4 (1)	17.0 (2)	23.8 (5)	26.7 (3)	
60	3.8 (1)	7.3 (1)	14.7 (5)	18.3 (5)	21.7 (5)	21.9 (5)
67	4.2 (1)	11.0 (1)	15.9 (1)	20.2 (5)	28.2 (4)	
68	9.2 (1)	17.2 (1)	23.7 (3)			
61	7.0 (1)	16.9 (1)	23.0 (4)			
51	4.3 (1)	5.4 (1)	6.2 (1)	10.5 (1)	14.8 (5)	
30	7.9 (1)	9.8 (1)	13.4 (2)			
70	4.8 (1)	13.0 (1)	22.5 (3)			
59	11.2 (1)	15.5 (3)				
21	4.4 (1)	9.3 (1)	13.9 (3)			

* The types of clinical courses are indicated by numerals 1 to 5 in parentheses adjacent to the percentage volume of cerebral damage.

TABLE 3—*Clinical Results of Increasing the Percentage Volume of Cerebral Damage by Producing Successive Cerebral Lesions at Intervals of Seventy-Two Hours (Exper 38, 20, 30, 37) and Ninety-Six Hours (Exper 22, 41, 39, 40)**

Experiment Number	Percentage Volume of Brain Lesion 1	Percentage Volume of Brain Lesions 1 and 2	Percentage Volume of Brain Lesions 1 to 3	Percentage Volume of Brain Lesions 1 to 4	Percentage Volume of Brain Lesions 1 to 5	Percentage Volume of Brain Lesions 1 to 6
38	5.5 (1)	13.5 (3)				
20	3.7 (1)	9.0 (1)	22.4 (5)			
30	5.0 (1)	10.9 (5)	16.7 (5)	23.6 (5)		
37	7.3 (1)	15.2 (2)	18.2 (5)	19.6 (5)	22.3 (5)	25.9 (5)
22	8.1 (1)	13.5 (1)	15.1 (1)	16.9 (1)	20.9 (5)	
41	4.6 (1)	10.8 (1)	14.3 (1)	18.4 (1)	19.6 (1)	26.8 (1)
39	8.2 (1)	13.8 (1)	23.3 (5)	27.5 (5)		
40	9.5 (1)	16.2 (1)	22.3 (5)	24.0 (5)	25.8 (5)	29.2 (5)

* The types of clinical courses are indicated by numerals 1 to 5 in parentheses adjacent to the percentage volume of cerebral damage.

Type 1 range, 3.7 to 26.8 per cent, average, 11.8 per cent

Type 2 one animal, 15.2 per cent

Type 3 one animal, 13.5 per cent

Type 4 no animal

Type 5 range, 10.9 to 29.2 per cent, average, 23.4 per cent

These studies indicate that the quantity of cerebral damage could be still further increased with survival of the animal if lesions were spaced at intervals of seventy-two or ninety-six hours rather than forty-eight hours. The principal effect of the more prolonged spacing, however, was to increase the number of animals surviving with the type 1 or type 5 clinical course and to decrease the number of animals following the fatal type 3 and type 4 clinical courses. It was apparent that still further cerebral ablation could have been accomplished had we attempted to maintain the nutritional state of the animals as they lapsed into the semi-stuporous vegetative state of the type 5 clinical course. If it is desirable for experimental purposes to do extensive cortical ablation without opening the skull and with a minimum mortality, the interval between operations should be at least ninety-six hours.

COMMENT

Three variables were most important in governing relationships between the percentage volume of cerebral damage and the clinical manifestations of animals. These variables were first, the percentage volume of acute cerebral damage, second, the percentage volume of cerebral damage due to previous injury and, third, the interval of time which elapsed between production of acute damage and preceding injury. When a minimum lethal volume of acute damage was produced at one operation, the animals usually soon recovered from the operation and after several hours of normal behavior lapsed secondarily into stupor and coma, terminated in a short time by convulsions and death. It was believed that the delayed onset of symptoms might be secondary to progressive pericapillary hemorrhage in the lesions, progressive edema in the area of cerebral degeneration or to the action of some toxic substance arising from disintegrating neural tissue. Postmortem studies failed to prove that the delayed onset of symptoms could be attributed to progressive pericapillary hemorrhage. Edema was apparent in and around the lesions, but measurement of the extent or the progression of this reaction was not possible. Even if it had been possible to measure accurately the increase of intracranial pressure, the measurements of pressure alone would not have distinguished between effects of edema and those of hemorrhage. No steps were taken to investigate the possible presence of a toxin liberated by the degenerating tissues.

It was believed that the role of edema might be more clearly defined if experiments could be done to take advantage of the fact that in the brain a hemorrhage continues to occupy space long after the edema which accompanies hemorrhage has subsided. For this reason, successive sublethal lesions were produced at intervals of twenty-four, forty-eight, seventy-two and ninety-six hours. These studies showed that when lesions were spaced at intervals of twenty-four hours, the mean survivable percentage volume of cerebral damage was not appreciably increased, so that the effect was the same as if the lesions had been produced simultaneously. However, when successive lesions were pro-

duced at intervals of forty-eight, seventy-two or ninety-six hours, the mean survivable percentage volume of total cerebral damage was greatly increased. The data indicated that a lapse of forty-eight hours after production of a near lethal volume of cerebral injury was sufficient to permit the reproduction of a second near lethal volume of cerebral injury without death. Increasing the interval of time from forty-eight to seventy-two or ninety-six hours seemed to have little additional effect. It was concluded that the factor responsible for this effect reached a maximum at about seven to ten hours after the production of an acute

TABLE 4—*The Maximum, Minimum and Average Percentage Volume of the Brain Occupied by Lesions in the Six Different Cerebral Locations in the Four Groups of Experimental Animals with Multiple Lesions Spaced at Intervals of Twenty-Four, Forty-Eight, Seventy-Two and Ninety-Six Hours*

Interval of Time Between Lesions	Size of Lesions	Per centage Volume of Brain Lesion 1	Per centage Volume of Brain Lesion 2	Per centage Volume of Brain Lesion 3	Per centage Volume of Brain Lesion 4	Per centage Volume of Brain Lesion 5	Per centage Volume of Brain Lesion 6
24 hours	Maximum	10.2	7.6	8.9	8.1		
	Minimum	1.3	0.2	1.3	2.0		
	Average	6.4	5.4	4.6	4.5		
48 hours	Maximum	11.2	9.9	9.5	8.2	8.0	0.2
	Minimum	3.8	1.1	0.8	3.6	3.4	0.2
	Average	6.0	5.5	5.6	5.8	4.7	0.2
72 hours	Maximum	7.3	8.0	13.4	6.9	2.7	5.6
	Minimum	3.7	5.3	3.0	1.4	2.7	3.6
	Average	5.4	6.8	7.4	4.1	2.7	3.6
96 hours	Maximum	9.5	6.7	9.5	4.2	4.0	7.2
	Minimum	4.6	5.4	1.6	1.7	1.2	3.4
	Average	7.6	6.0	5.2	3.0	2.3	5.3

lesion and that it persisted thereafter for at least twenty-four hours and disappeared rapidly in the interval between twenty-four and forty-eight hours. It is assumed, without complete proof, that the factor was probably congestion and edema in and around the lesion. At least, the curve of degree of influence of this factor in time has been established.

The percentage volume of cerebral damage due to previous injury influenced the clinical course of animals in other ways than by its contribution to the lethal effect of additional injury. This was apparent in animals of all groups. Increments of cerebral damage spaced at intervals of twenty-four hours led to the frequent appearance of a type 5 clinical course, which was rarely encountered in animals with a similar volume of cerebral damage produced at a single operation. This clinical course, characterized by semistupor and lack of ambition or desire to eat, was usually terminated by a small increment of cerebral damage

produced by the next operation. After the final operation the animals pursued the type 4 clinical course and died after several hours without a postoperative return of consciousness. This same trend of conversion from a type 1 or a type 2 to a type 5 clinical course was still more pronounced when increments of cerebral damage were spaced at intervals of forty-eight hours and most pronounced when the interval of time between operations was seventy-two or ninety-six hours. The onset and persistence of the type 5 clinical course seemed to be more closely correlated with progressive subacute cerebral degeneration and widespread cortical inactivation than with the actual percentage volume of cerebral damage, although most animals had a volume of cerebral injury in excess of that which an animal could survive at a single operation.

It may be permissible to compare the findings in the experimental animals with findings in human patients who have acute lesions of similar nature in the cerebrum or in intracranial neoplasms. Certain types of human cerebral infarction or cerebral hemorrhage of non-traumatic origin closely resemble the experimental lesions. As a rule human cerebral vascular accidents of the relative magnitude of the experimental lesions lead promptly to unconsciousness. Experimentally, this symptom was delayed in onset unless the percentage volume of acute cerebral damage was very large and essentially beyond limits of survival or unless the preexisting percentage volume of cerebral damage was sufficient to create a persistent semistuporous condition, represented by the type 5 clinical course. Furthermore, most people with lesions of the magnitude produced experimentally exhibit localizing neurologic signs. Localizing signs were not detected in the animals, nor were they expected, because the cortical areas and tracts concerned with motor function in rabbits are different anatomically from those in man. Most persons with lesions of the relative magnitude and location of the experimental lesions improve rather rapidly or die within twelve to seventy-two hours after the onset of symptoms. There was considerable similarity in this respect between the usual clinical course of patients and that of animals with sufficient cerebral damage to cause symptoms. We have found that when single or multiple simultaneous acute lesions were produced in a previously undamaged brain, the development of significant symptoms occurred either in the first few postoperative hours or not at all. If significant symptoms developed, death, with rare exceptions, occurred in twenty-four hours. However, if acute lesions were produced in a brain already damaged by previous lesions, there was a pronounced tendency for animals to remain semistuporous or comatose for several days. The course of these animals more closely resembled the lingering, prolonged comatose state of some

patients with cerebral infarction or hemorrhage. Some of these animals might have recovered had their nutritional needs been cared for sufficiently well, but they seemed to be in an irreversible vegetative state.

There is little need to discuss in detail the gross and microscopic similarities between many human lesions and those produced experimentally. Sudden focal cerebral necrosis with variable well restricted hemorrhage within the cerebrum and an accompanying edema within the area of necrosis and neighboring gray and white matter are conspicuous features of the human and experimental lesions. Resolution and repair, as previously described, are nearly identical in both processes. In our opinion, the similarities are sufficient to justify an attempt to establish by quantitative experimental methods a sound basis for treatment of pertinent types of acute expanding intracerebral lesions in man.

SUMMARY

Previous studies have shown that acute closed cerebral lesions characterized by edema, hemorrhage and necrosis could be reproduced quantitatively in rabbits by a standard hypothermal method. The average minimum lethal amount of cerebral damage was 14.3 volumes per hundred volumes of the brain, and no animal survived cerebral damage in excess of 18.5 per cent of the brain. The present experiments have shown that if acute cerebral lesions of sublethal dimensions were produced successively in the same animal at intervals of twenty-four hours, no increase in the average minimum lethal percentage volume or maximum survivable percentage volume of cerebral damage occurred. However, if acute cerebral lesions were produced successively in the same animal at intervals of forty-eight hours, the average minimum lethal percentage volume and maximum survivable percentage volume of cerebral damage were increased by about 40 to 50 per cent. When the interval between operations to produce successive lesions was prolonged to seventy-two or ninety-six hours, the results were about the same as those obtained when the interval was forty-eight hours. The survivable magnitude of cerebral ablation by these methods was about 50 per cent of the cerebrum or 30 per cent of the brain. These data indicated that factors responsible for death from acute cerebral injury of this type had not begun to subside until twenty-four hours had elapsed but had largely disappeared by the end of forty-eight hours. There were reasons for believing that cerebral edema was the most important of these factors.

Clinical observations indicated that most animals with an acute lethal lesion produced at a single operation had a period of several hours of normal postoperative behavior before the onset of stupor, coma and convulsions followed by death. When a similar or greater total volume

of cerebral damage was produced by acute lesions made successively at intervals of twenty-four, forty-eight, seventy-two or ninety-six hours, the animals showed a pronounced tendency to lapse into a stuporous irreversible vegetative state without localizing neurologic signs. This seemed to be related to a combination of progressive cerebral degeneration and total percentage volume of cerebral damage.

These observations, correlating symptomatic course with quantity, location and age of closed cerebral lesions, define experimental conditions which should prove useful in validation of therapy of acutely expanding intracerebral lesions characterized by edema, hemorrhage and necrosis.

INTESTINAL EMPHYSEMA IN INFANTS

A Review of the Literature, with Seventeen New Cases Reported

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INTESTINAL emphysema of infants (i e, emphysema intestinalorum, gas cysts of the intestine, diffuse emphysema of the intestinal wall, multiple cysts of the intestine), first described as a clinical entity by Bang,¹ in 1876, has been infrequently described in the literature. The term "pneumatosis cystoides intestini" has been used by some writers, and although not standard nomenclature it seems descriptive and inclusive. Jackson² collected 171 cases of this disease from the world literature and added one of his own in which an infant was involved, making a total of 12 cases in which the condition was observed in an infant. Lindsay and associates³ described a case in detail in 1940. The apparent obscurity of this disease, coupled with the curious fact that 17 cases were observed during the period from January 1945 to September 1947 at the Children's Hospital Washington, D C, dictates the adding of these cases to the literature.

INCIDENCE AND PATHOGENESIS

The sporadic nature of the disorder has been noticed by others. An incidence of 4 cases in 260 autopsies, all occurring in one year, was noted by Moore.⁴ Five of the 6 cases of Botsford and Krakower⁵ were also observed within one year. Sex, race, climatic conditions, the season of the year and geography have not been shown to be definitely correlated with the incidence.

From the Department of Pathology, Children's Hospital

1 Bang, B L F. *Nord med ark* **8** 18, 1876

2 Jackson, J A. *Surg, Gynec & Obst* **71** 675, 1940

3 Lindsay, J V, Rice, E C, and Selinger, M A. *Arch Path* **30** 1085, 1940

4 Moore, R A. *Am J Dis Child* **38** 818, 1929

5 Botsford, T W, and Krakower, C. *J Pediat* **13** 185, 1938

The cause is obscure, investigation having failed to establish a definite etiologic factor. The coexistence of other diseases, notably colitis, ulcer, gastric carcinoma, appendicitis, strangulated hernia and parasitic infections, has been noted. Primary pneumatosis can occur, but most commonly it is secondary, associated with a basic gastrointestinal disorder.

Various theories have been advanced, with (1) the bacterial and (2) the mechanical the most prominent. In addition Menna⁶ postulated (3) neoplasm, (4) hematoma, (5) local change in fat metabolism and (6) parasitosis as causal conditions. The bacterial theory subscribes in part to the idea that bacterial action produces gas in the tissues of the wall of the intestine. No conclusive bacteriologic studies have been reported, although various authors have described bacteria observed in the cysts or their vicinity either of a specific nature or of the variety normally found in the intestine. The latter are thought to invade the lymphatic channels under certain conditions. The clinical picture is not one of a gas-forming infection, and the gas recovered from the cysts contains mostly oxygen, which is not in keeping with bacterial infections.

The mechanical theory states that an increase in the intraluminal pressure occurs, with a break in the mucosa. The latter is due to gaseous overdistention or localized infection of the mucosa. With hyperperistalsis or obstruction the gas is forced through the defective mucosa and accumulates in the various layers and beneath the serosa. It may often spread through the lymphatic channels or along the peritoneal planes. Various authors have expressed the belief that the gas is disseminated into the tissues rather than that it spreads exclusively by way of lymphatic channels.

CLINICAL FINDINGS

The patients do not exhibit symptoms which can be directly referred to pneumatosis, and the diagnosis is made usually after death. The infants are in poor general condition. Some observers, notably von Hacker⁷ and Coelho,⁸ described the sensation of crepitation on palpation of the abdomen, with a peculiar tympanism on percussion. The abdomen is usually soft and compressible. The distended bowel loops may feel like tumor masses, although the size varies from day to day. These signs vary with the location of the gas and the extent of involvement. The symptoms of gastritis, ulcer, stenosis, etc., all normally overshadow signs of pneumatosis. Fever and symptoms of an infection of the respiratory tract are common.

6 Menna, L. *Policlínico (sez. chir.)* **47** 220, 1940.

7 von Hacker, cited by Memm, R. *Policlínico (sez. chir.)* **141** 408, 1934.

8 Coelho, N. A. *Ann. Fac. de med. de São Paulo* **15** 201, 1939.

European literature, and recently American reports, have contained descriptions of the roentgen aid to diagnosis. Matranola⁹ described a typical roentgenogram, which he considered pathognomonic, accompanied by symptoms of pyloric stenosis. Learner and Gazin¹⁰ stated that translucent areas of gas are seen in the contour of an otherwise normal bowel and that when a contrast medium is used there is an inability to fill the lumen, giving the impression of filling defects. Areas of decreased density are observed between the contrast material and the outer intestinal wall. The presence of this inconsistent filling defect of increased translucency is considered pathognomonic by Learner.

CASES OBSERVED

During the period from January 1945 to September 1947, at the Children's Hospital, as stated, 17 infants under 8 months of age were shown at autopsy to have intestinal emphysema (table). Five hundred and one autopsies were performed within the same period. Ten of

Statistical Information

Year	Cases	Male	Female	White	Negro	Age, Months				
						1	2	4	6	8
1945	10	5	5	7	3	3	4	1	1	1
1946	5	3	2	2	3	2	0	2	1	0
1947	2	2	0	2	0	1	1	0	0	0
Total	17	10	7	11	6	6	5	3	2	1

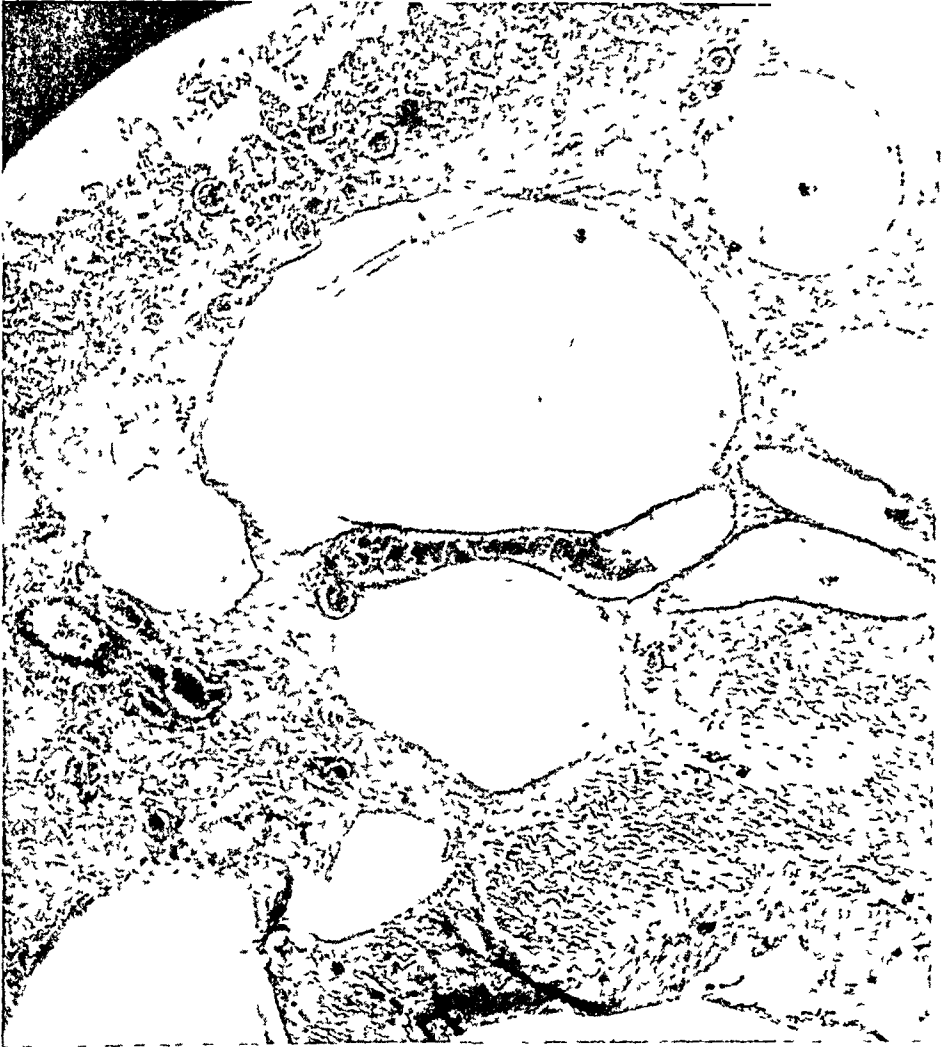
the infants died in 1945, 5 in 1946 and 2 in the first nine months of 1947. No cases of the disease have been seen since that time. Eleven of the infants were white and 6 were Negro. Ten were males and 7 were females. Nine (54 per cent) of the patients were observed in the months of March and April.

It is evident from the various theories that the pathogenesis is uncertain. The mechanical theory seems most likely, although attempts to produce pneumatosis experimentally by injecting air into the peritoneal lymphatic vessels have been unsuccessful. For pathologic changes to be present, ulcerative gastrointestinal lesions occurring concomitantly seem to be necessary. Thirteen of our 17 patients had ulcerative ileocolitis, while the remaining 4 had various stages of ileocolitis. While a basic bacterial factor appeared to be combined with mechanical factors in many instances, bacterial cultures, both ante mortem and post mortem, were not significant. Diarrhea was present in a majority of the cases, but except for the recovery of Salmonella in 2 instances bacterial

9 Matranola, cited by Memmi, R. Policlinico (sez. chir.) **141**: 408, 1934.
10 Learner, H. H., and Gazin, A. Am. J. Roentgenol. **56**: 464, 1946.

data were insignificant. The cause of pneumatosis of the intestines probably is a combination of the two types of factors with which the major theories are concerned.

All of our patients had temperatures ranging up to 105 F. Fifteen had infection of the respiratory tract during some stage of their illness. The 2 remaining children had severe infectious diarrhea.



Photomicrograph showing cystic spaces in the submucosa and muscularis of the colon, $\times 50$

Vomiting and diarrhea were prominent. All but 2 patients showed a loss of weight during the period of hospitalization.

Despite the awareness of the existence of this disease, none of the clinical signs described by others were definitely ascertained to be present in these cases. In 1 case the diagnosis was substantially championed before death, but in that case the infant, moribund, was seen late in the

year 1945, when circumstantial evidence pointing to the diagnosis was most prominent. Clinical impressions failed to warrant the taking of roentgenograms in our cases. Usually the basic pathologic observations caused attention to be diverted into other channels.

THE LESIONS

The lesions described in the literature had a predilection for the distal part of the ileum, with the cecum, ascending colon and jejunum, in that order, most frequently involved.¹¹ Rarely the stomach, the omentum and the gastrohepatic ligament have been involved. In our series the ileum was involved in 12 cases, the cecum in 10, the ascending colon in 7, the jejunum in 5, the transverse colon in 2 and the descending colon and the mesentery of the upper part of the ileum in 1. Masson¹² previously stated that the transverse and descending portions of the colon are spared. Grossly, the intestinal wall is found to be involved with air-containing cysts varying from pinpoint size to pea size. Rarely they may be larger. The cysts are noted in all layers of the intestines. When situated beneath the serosa the affected segment of the intestine shows multiple cysts. In gross appearance the intestine involved is like "sponge rubber."

Microscopically, the cystlike spaces contain gas and occasionally a protein-like material. There is no obvious endothelial lining, and the spaces resemble ruptured lymph spaces. There are varying degrees of inflammatory changes with hemorrhage, thrombus formation in the smaller blood vessels and edema (figure). Moore⁴ noted that in children the predominant location of the cysts was in the mucosa and submucosa, in contradistinction to adults, in whom the serosa was most commonly involved. He found giant cells infrequently and only with smaller cysts in association with fibrosis and fibrin deposits.

COMMENT

The condition described here actually has minor clinical significance. The sporadic occurrence has little correlation with race, sex, season or geography, although it is interesting that cases are observed almost always in groups. The condition probably has been recognized more often than has been reported and when noted has been regarded as insignificant by the observers. It seems probable that the increased use of sulfonamides and antibiotics, along with the lowered incidence and lessened severity of infections of the gastrointestinal and respiratory tracts, has decreased the incidence of pneumatosis cystoides intestini.

11 Urban, H. *Forsch. a. d. Geb. d. Röntgenstrahlen* 55:231, 1937. Bang¹ Jackson² Lindsay and others³ Moore⁴ Botsford and Krakower⁵ Menna⁶ von Hacker⁷ Coelho⁸ Matranola¹⁰

12 Masson, P. *Ann. d'anat. path.* 2:541, 1925.

The pathogenesis is probably described best by a combination of the two major theories stated. It is our belief that the condition is always associated with an alteration in the mucosa allowing entrance of bacteria and gas, aided by peristalsis and/or spasm of the intestine. The bacteria after entering the wall of the intestine may produce gas in some instances. The gas then penetrates the breaks in the mucosa traversing the lymphatic channels and extends directly along tissue planes.

The most common bacteria obtained by culturing rectal material before death and material from the lesions after death are of species not likely to cause the type of tissue destruction noted in pneumatosis. Pathogenic organisms, notably hemolytic *Staphylococcus albus*, were recovered in 3 cases and *Salmonella enteritidis* in 2. Although severe ulcerative ileocolitis is not uncommon in the patients, similar ulcerative lesions occur commonly unassociated with pneumatosis. Definite proof of the duration of pneumatosis cannot be given, but the pathologic alterations seem older than a few hours and are not due to postmortem changes.

SUMMARY

The infrequent, sporadic condition, emphysema of the intestine, is discussed. The pathogenesis is obscure and a combination of the two major theories, bacterial and mechanical, is advanced as depicting the probable cause.

Clinical and roentgen examination appear to be of little value in making a diagnosis, and a definite diagnosis is usually made only at necropsy.

The typical gross appearance of the involved intestine is that of "sponge rubber," and the microscopic picture is that of gas cysts of the intestinal wall.

Seventeen new cases are presented, which, with those previously reported, bring the total number of reported cases to 30.

A brief review of the literature is included.

NORTH AMERICAN BLASTOMYCOSIS

Report of a Case in Which a Patient with Meningeal Involvement was Treated
with Streptomycin and Promin®

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NORTH American blastomycosis is a chronic specific fungous disease caused by *Blastomyces dermatitidis*. Two clinical forms are recognized, cutaneous and systemic. The cutaneous form typically is manifested by a solitary chronic or subacute ulcerating lesion, and secondary systemic involvement is rare. Systemic blastomycosis frequently involves the skin secondarily, and often the patient has widespread involvement of many internal organs. The lung is a focus in approximately 96 per cent of the cases, and the spleen, liver, bones, prostate and central nervous system may show involvement.

Involvement of the central nervous system is rare, as indicated by the fact that in 1939 Martin and Smith¹ were able to find only 16 acceptable cases reported in the literature. Although intracranial lesions are more common, occasionally the spinal cord may be attacked.² Meningeal involvement is most uncommon. In a review of the literature to 1945, Friedman and Signorelli³ found less than 10 acceptable cases. Our review of the English literature has revealed 4 additional acceptable reported cases.⁴

Although a presumptive diagnosis of blastomycosis can be made on the basis of the clinical picture, it is necessary to resort to laboratory tests to make an unequivocal diagnosis. The organism must be identified in a direct smear of material from the available lesion, it must be grown on suitable culture mediums, and tissue sections must show

From the Laboratory Service, Veterans Administration Medical Teaching Group, Kennedy Hospital.

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1 Martin, D. S., and Smith, D. T. *Am Rev Tuberc* **39** 375, 1939.

2 (a) Stober, A. M. *Arch Int Med* **3** 509, 1914. (b) Wade, W. H., and Bel, G. S. *ibid* **18** 103, 1916.

3 Friedman, L. T., and Signorelli, J. J. *Ann Int Med* **24** 385, 1946.

4 (a) Gaspare, I. *Arch Neurol & Psychiat* **22** 475, 1929. (b) Littman, M. L., Wicker, E. H., and Warren, A. W. *Am J Path* **24** 339, 1948. (c) MacBride, C. M., and Thompson, E. I. *Arch Dermat & Syph* **27** 49, 1933. (d) Wilhelmj, C. M. *Am J M Sc* **169** 712, 1925.

the characteristic histopathologic appearances.⁵ Baker⁵ expressed the belief that a biopsy specimen showing the characteristic tissue is sufficient for absolute diagnosis. He has described in detail the histopathologic observations characterizing blastomycotic infection. The lesions are essentially the same, regardless of the organ in which they occur. The typical lesion consists of miliaiy abscesses and granulomatous tissue containing blastomycetes, polymorphonuclear granulocytes (predominantly neutrophils), plasma cells, epithelioid cells, giant cells and necrotic debris. The presence of miliaiy abscesses and the predominance of polymorphonuclear cells, within these abscesses, help to differentiate blastomycosis from tuberculosis even in the absence of demonstrable specific organisms. In bone the lesion starts in the epiphyseal region and is exceedingly destructive.⁶ Lesions of the skin show, in addition, marked epithelial hyperplasia, so that occasionally the lesion may be confused with epidermoid carcinoma.³ However the microscopic intraepidermal abscesses which are usually present in blastomycosis aid in differentiating the two conditions.

Treatment of the systemic form of infection by the classic means of roentgen rays, iodides and desensitization has been generally unsuccessful. Herrell and associates⁶ and Meyer and Ordal⁷ have found penicillin ineffective. Following is the report of a case in which streptomycin and promin[®] (sodium-p,p'-diaminodiphenylsulfone-N,N'-dioxetose sulfonate) failed to alter the course of the infection.

REPORT OF CASE

A 55 year old Negro man was admitted to the Veterans Administration Hospital, Jan 1, 1948, with a diagnosis of chronic suppuration of the lower lobe of the right lung and secondary anemia. There was a history of an acute onset of illness in July 1947, with distress in the right upper quadrant of the abdomen, vomiting and high fever. These symptoms persisted until the patient was admitted to a hospital Oct 1, 1947, where a seven day course of penicillin was given. During the subsequent three months, he had episodes of fever, the temperature varying from 99 to 102 F, and occasionally brought up bloody sputum, although at this time he had no pain, chills or foul expectoration. He stated that he had lost 40 pounds (18 Kg) during the two months prior to admission to the Veterans Administration Hospital.

On examination the patient was seen to be a well developed, well nourished Negro man, not appearing acutely ill. Positive findings were moderate guarding of the muscle and tenderness in the right upper quadrant of the abdomen and two small papular eruptions of the skin of the face. Examination of the lungs disclosed no abnormal condition.

On admission the blood count showed 3,800,000 red blood cells and 17,500 white cells per cubic millimeter. The hemoglobin content was 111 Gm per

5 Baker, R. D. *Am J Path* **18** 479 1942. *Arch Path* **44** 439 1947.

6 Herrell, W. E., Nichols, D. R., and Heidman, D. H. *J A M A* **125** 1003, 1944.

7 Meyer, E., and Ordal, Z. J. *J Infect Dis* **79** 199, 1946.

hundred cubic centimeters. The differential white cell count showed neutrophils 76, lymphocytes 22, monocytes 1 and eosinophils 1 per cent. The sedimentation rate was 32 mm per hour. Urinalysis gave results within normal limits. No parasites or ova were found in the stool specimens. Repeated examinations of sputum and gastric washings showed no acid-fast organisms with the exception of one report of a very rare acid-fast bacillus on direct smear. All cultures were negative for tubercle bacilli. Roentgen examination of the chest, Jan 2, 1948, revealed, in the lower lobe of the right lung, an inflammatory process approximately 7 by 7 cm. A second roentgenogram, February 2, revealed in addition a bilateral infiltrative process suggestive of pulmonary edema.

Because of the roentgen findings and the one questionable positive result of an examination of sputum, tuberculosis was suspected. January 10, signs of minimal meningismus and occipital headache developed. Kernig's sign could be elicited, and a temperature of 103 F was recorded. On repeated spinal taps the pressure of the spinal fluid varied from 100 to 560 mm of water. The fluid was constantly cloudy. The white blood cell count varied from 719 to 1,266 per cubic millimeter, 51 to 91 per cent being polymorphonuclear neutrophils. Total proteins were elevated, while the sugar and the chlorides were at normal levels. The test for globulin was positive and the Kolmer test negative.

Because of the probability that the patient had tuberculous meningitis, on Jan 10 treatment was begun, consisting of streptomycin, 2 Gm, intramuscularly, and promin,[®] 4 Gm, intravenously, daily and penicillin, 15,000 units, with streptomycin, 0.05 Gm, intrathecally, every two days. After one week of this regimen the patient's condition had not improved. By January 24 he had episodes of stupor and disorientation. At this time he was given sulfadiazine, 0.75 Gm every four hours, and sulfamerazine, 0.75 Gm every eight hours. No improvement occurred. February 5 the sulfonamides were supplanted by penicillin, 10,000,000 units daily for three days. The patient became quite stuporous and completely disoriented, and on February 18 all specific therapy was discontinued.

February 19, a smear of material from the small papillomatous lesion over the dorsum of the nose revealed a budding fungus characteristic of *Blastomyces dermatitidis*. A skin test for blastomycosis was negative. Potassium iodide therapy was begun. February 23, the patient's temperature rose to 104 F, his pulse rate to 105 per minute and respirations to 60 per minute, and the patient died.

At necropsy, three hours after death, the pertinent findings were confined to the lungs, the meninges and a small lesion of the bridge of the nose.

The left and right lungs weighed 710 Gm and 630 Gm, respectively. Their surfaces were smooth and glistening, and the entire lung parenchyma had reduced crepitaney and finely nodular consistency. On section, the primary, secondary and tertiary bronchi were partially filled with a frothy reddish fluid, and a similar fluid exuded from the cut surface. No nodules could be seen on the cut surface.

The brain weighed 1,320 Gm, and the gyri were moderately flattened. All the structures about the base of the brain, from the optic chiasma posteriorly to the posterior part of the pons and including the cerebellar tonsils, were covered by yellowish gray granulomatous plaques, 2 to 4 mm in thickness. Serial section of the brain revealed moderate bilateral symmetric dilatation of the lateral ventricles and a dilated cavum of the septum pellucidum. The third and fourth ventricles were also dilated. This internal hydrocephalus was the result of the fibrous adhesions and the granulation tissue which were abundant in the region of the foramina of Magendie and Luschka. The cut surface of the brain showed congestion of small blood vessels, most marked in the region of the pons.

The lesion over the dorsum of the nose was circular and measured 1 cm in diameter. It was covered by a dry scale, and its borders were not raised.

On histologic examination the parenchyma of the lung showed many foci of granulomatous reaction, which varied from 0.5 to 2 mm in diameter. These foci showed necrosis and suppuration and consisted of polymorphonuclear cells,



Fig 1—A granulomatous exudate extends from just anterior to the optic chiasma caudad over the cervical cord.

necrotic debris, epithelial cells, fibrous connective tissue and an occasional large multinucleated foreign body giant cell. Occasional round or oval yeastlike bodies 10 to 15 microns in diameter and with double-contoured, highly refractile walls were seen, and these were particularly prominent within the giant cells. The alveolar structure of the lung was intact except where replaced by these granulomatous lesions. Within the lumens of the alveoli considerable edema



Fig 2—Upper part There are infiltration and thickening of the meninges by granulomatous tissue over the pons. The lateral and third ventricles are dilated, and there is splitting of the septum pellucidum due to dilation of the cavum.

Lower part Granulation tissue from the meninges. Note three organisms in the center of the section, one of which is just separating by budding. $\times 450$

fluid was present. As to the brain, except for congestion of small blood vessels, representative sections revealed no changes. The granulation tissue, previously mentioned, was closely adherent to the surface of the organ but did not extend into it. Here a granulomatous reaction was present, similar to that observed in the lung, although there were more milary abscesses, giant cells and organisms than in the pulmonary lesions. Sections from the meninges showed more organisms to be forming buds than did sections from the lungs. A smear of material from the lesion of the nose revealed blastomycotic organisms, and smears and cultures of material from the lungs and meninges confirmed the diagnosis of blastomycosis.

COMMENT

The marked involvement of the lungs and the meninges places this case in the group of cases of systemic blastomycosis. The small superficial cutaneous lesion, in all probability, is merely another focus of the systemic disease. This lesion developed late in the course of the disease and neither was as extensive as, nor had the clinical appearance of, the lesions seen in cases of the cutaneous form of blastomycosis.

There are several possible sources for the meningeal involvement. One is the primary pulmonary focus, with a hematogenous spread. A second is the lesion of the bridge of the nose, with the infection extending to the meninges by lymphatic and venous return. Which of these mechanisms was responsible for the meningeal involvement in this instance could not be determined. Friedman and Signorelli³ in reporting a similar case pointed out that if one accepts the premise of a hematogenous spread it would be difficult to explain why the meninges, which are infrequently involved, should be attacked while other organs and tissue which are more frequently involved should be spared.

Wilhelmj^{4a} described a primary meningeal form of systemic blastomycosis, in which the meninges alone show involvement. He suggested that in this form the meninges are infected during the initial period of invasion and death occurs before the general metastatic foci appear. The portal of entry, presumably, would be pulmonary.

The therapeutic failure of penicillin and streptomycin is consistent with the findings in previous investigations.⁸ However, this probably represents the first clinical trial of streptomycin and promin[®] in systemic blastomycosis.

SUMMARY

The literature of systemic blastomycosis with meningeal involvement is reviewed, and an additional case, in which the patient was unsuccessfully treated with streptomycin and promin,[®] is reported in detail.

8 Herrell and others⁶ Meyer and Ordal⁷ Stober^{2a}

FIBROSARCOMA OF THE HELIX OF THE EAR

BÉLA HALPERT, M D

AND

VICTOR C HACKNEY, M D

OKLAHOMA CITY

SARCOMA of the external ear is rare Hand and O'Connor¹ in 1938 recorded a myxofibrosarcoma of the ear of a 5 year old girl They found that only 2 cases of fibrosarcoma of the external ear had been reported in the preceding decade one by Treer and Kallo,² the other by Speziale³ No additional reports have since appeared In this paper 2 recently observed cases of fibrosarcoma of the helix are presented

REPORT OF CASES

CASE 1—J D, a 71 year old white man, was first seen at the University of Oklahoma Hospitals Dec 18, 1946 He stated that two months previously a small, nontender nodule appeared on the anterior and superior surface of the left ear This progressively enlarged A biopsy of the lesion made in the outpatient department revealed a cancer He was admitted to the hospital December 31 Examination revealed a round purple mass, 3 cm in diameter, pedunculated, soft, on the anterior superior surface of the left auricle The site of the excision of biopsy tissue was not healed The growth appeared not to involve the subjacent cartilage The regional lymph nodes were not palpably enlarged There were no other pertinent findings Resection of most of the left auricle was performed by Dr Harrell C Dodson Jr on Jan 3, 1947 There was no evidence of recurrence of the growth when the patient was seen Dec 6, 1948

The specimen consisted of the upper two thirds of the left auricle On the upper anterior portion of the helix was a soft mass 2.5 cm in diameter, partly covered by a black crust The growth was composed of homogeneous soft yellow-white tissue, involving only the soft parts and not extending to or invading the cartilage Microscopic preparations (fig 1) stained with hematoxylin and eosin revealed round, oval and cigar-shaped nuclei in a hardly discernible cytoplasm that faded into a delicate fibrillar ground substance The cells were arranged in streams and whorls, in places closely packed, elsewhere farther apart There was marked variation in the size and the shape of the cells, occasional cells had giant proportions, and many were seen in a state of division Large, thin-walled

From the Department of Pathology, University of Oklahoma School of Medicine, and the University of Oklahoma Hospitals

1 Hand, F, and O'Connor, G B Ann Otol, Rhin & Laryng **47** 1096, 1938

2 Treer, J, and Kallo, A Orvosí hetil **72** 1128, 1928

3 Speziale, V Arch ital di otol **43** 526, 1932

blood spaces were numerous. There were occasional small areas of hemorrhage and necrosis. The growth extended to the covering epithelium, in places leaving it intact.

CASE 2—A. B., a 92 year old white man, was admitted Sept. 1, 1948. He stated that two months previously, following trauma, a small, nontender mass appeared on the superior surface of the auricle of the right ear. The mass gradually enlarged. About two weeks before admission, a small portion of skin over the growth became ulcerated. Examination revealed a mass 2.5 cm in diameter, covered with a yellow-brown crust and elevated 2 cm above the cutaneous surface of the helix of the right ear. The growth was not movable over the

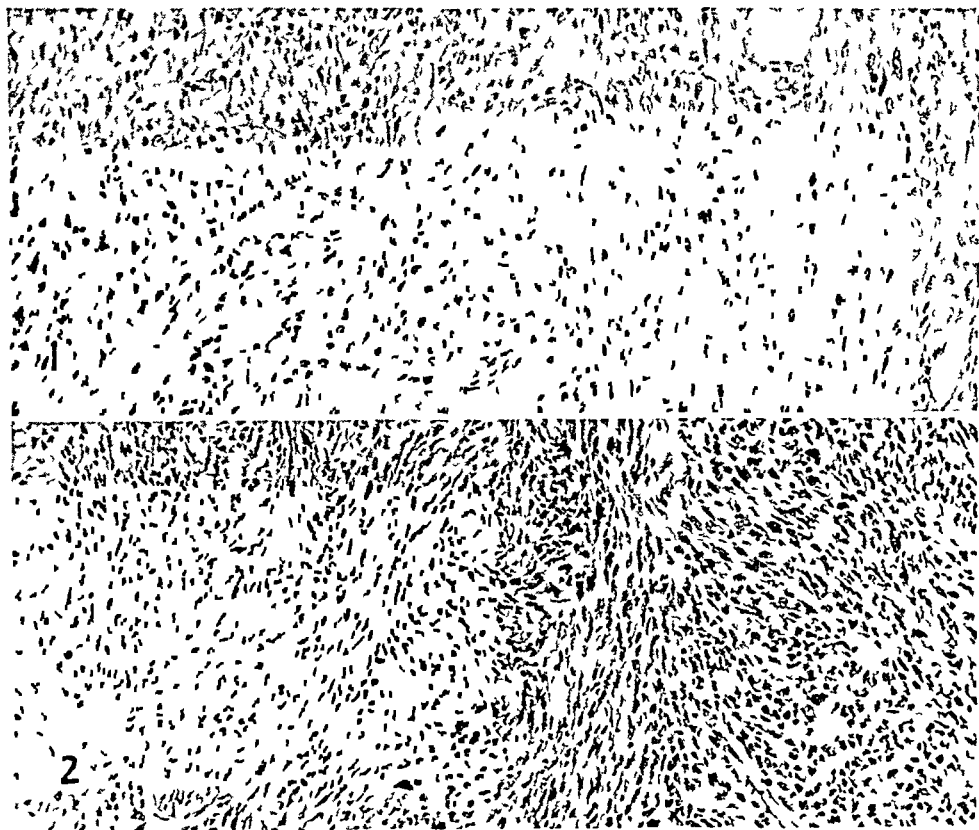


Fig 1—Microscopic structure of the growth in case 1, $\times 100$

Fig 2—Microscopic structure of the growth in case 2, $\times 100$

underlying cartilage. The regional lymph nodes were not palpably enlarged. There were no other pertinent findings. Resection of the upper third of the auricle of the right ear was performed by Dr. John H. Clymer September 7. The operative wound was well healed when the patient was last seen October 14.

The specimen consisted of the upper third of the right auricle. On the superior posterior surface of the helix was a firm gray-red mass, 3 by 2 by 2 cm. Most of the surface of the mass was crusted. The cut surfaces were light gray and had a fish meat appearance. The growth did not involve the cartilage. Microscopic preparations (fig 2) stained with hematoxylin and eosin disclosed neoplastic cells with round or elongated, spindle-shaped nuclei in a stream and whorl-like

heard The Wassermann reaction of the blood was strongly positive Induction of labor was started on the day after admission, and a dead girl was delivered on the third day, after passage of 35 liters of amniotic fluid The Wassermann test of the amniotic fluid and of the cord blood was negative

The fetus measured 35.5 cm in length and weighed 1,080 Gm The whole body, especially the face and the upper extremities, showed marked pitting edema The abdominal cavity contained 20 cc of clear yellowish fluid The peritoneal surfaces were smooth and glistening The left side of the diaphragm protruded below the costal margin, while the right dome was at the level of the fifth rib



Fig 1—Tumor of the lower lobe of the left lung, the upper lobe, the heart and the aorta, and the right lung, viewed from behind The upper lobe of the left lung has been displaced backward and downward

The left thoracic cavity was occupied by a mass which pushed the diaphragm downward and displaced the mediastinum to the right side Owing to cardiac displacement, the venae cavae were compressed The mass represented the malformed lower lobe of the left lung The lower branch of the left bronchus led into the mass for a short distance The mass measured 8.5 cm longitudinally, 6 cm transversely and 4 cm anteroposteriorly It had many congested blood vessels under its pleural surface and was rather soft in consistency Its cut surface presented a solid, grayish white tissue with many cysts of various sizes and shapes Cysts scattered throughout the mass varied from 2 mm to 15 cm in



Fig 2—*A*, tumor showing large tubules (bronchioles) lined by pseudostratified columnar cells, similar to those of normal bronchiolar mucosa, and small tubules (terminal bronchioles) lined by cuboidal cells. These two types of tubules make up the bulk of the tumor. The overgrowth of the bronchioles, especially of the terminal bronchioles, is obvious, and the tumor may be regarded as a hamartoma $\times 85$.

B, peripheral portion of the malformed lower lobe of the left lung, showing malformed tissue or hamartoma in the upper half of the field and normal lung tissue in the lower half $\times 85$.

diameter The largest was 4.5 cm in its longest diameter The cysts did not communicate with the left lower bronchus, but frequently with one another through small circular channels The right lung was displaced, compressed and atelectatic, and congested A small amount of clear yellowish fluid was in the left pleural cavity, none, in the right cavity The other organs showed no remarkable changes or cysts The placenta was disproportionately large (21 by 17 by 4 cm), thick and edematous, weighing 650 Gm

Sections from the malformed lower lobe of the left lung revealed numerous small cystic spaces lined by a single layer of cuboidal epithelium and surrounded by scanty fibrous stroma These structures apparently represented much dilated terminal bronchioles Scattered among them were a smaller number of much larger, irregular-shaped cystic spaces lined by pseudostratified columnar epithelium, often ciliated, and surrounded by a basement membrane with a mantle of well developed smooth muscle These apparently represented the larger bronchioles, but no cartilage or mucous glands could be found in their walls In places a third type of glandular structure was observed, lined by a single layer of tall columnar epithelium with clear cytoplasm and basally situated nuclei, exactly similar to the epithelium of the stomach and the colon It was surrounded by a basement membrane but was devoid of smooth muscle Lastly, normal lung tissue was seen at the periphery (i.e., adjacent to the pleura) Between the malformed and the normal tissue there was no clear demarcation or fibrous septum

Sections from the upper lobe of the left lung and from the right lung showed normal tissue only

In the liver there was dilatation of the central veins, also pronounced hemopoiesis, indicating prematurity The chorionic villi of the placenta showed capillary engorgement and edema

The other organs revealed no significant changes

REVIEW OF THE LITERATURE

As stated, 10 cases of a lesion of the lung similar to ours were found by a search of the available literature (table) What Nordmann^{2g} called an "accessory lung" revealed histologically the same structure as the lungs showing adenomatoid malformation

In 4 of the reported cases the subject was a male infant, in 5 a female infant and in 1 the sex was not specified Almost all the subjects were premature infants, many stillborn

The lesion involved only one lobe in 6 cases, it involved two lobes in 2 cases and the entire right lung in 1 case In the remaining case (Sternberg's^{2d}), which must be considered as an exception, both lungs were diffusely involved and symmetrically enlarged Otherwise the left lung was involved 5 and the right 4 times The lesion was a diffuse metamorphosis of an entire lobe (or lung) with a smooth contour The bronchus leading to the lobe ended blindly or could not be further traced when its widened ramifications were reached (Koboth²ⁱ) It was atretic and lacked cartilage in Meyer's^{2e} case

Microscopically, all the involved lobes revealed large and small cystic spaces, lined by columnar and cuboidal epithelial cells The larger cysts had elastic fibers and smooth muscle in the walls Absence of cartilage was noted by Koboth and practically all the other authors (Esch,^{2h} Werbter,^{2f} Meyer,^{2e} Seyffert,^{2c} Stork¹ and Lahm^{2b}), while Koboth further noted absence of arteries and Lahm absence of mucous glands Koboth noted the presence of tall columnar "gastric" epithelium, even with polypoid formations

The lesion was called by various names "Cystic malformation and excessive overgrowth of the bronchial system" (Stork), "congenital adenoma of the small bronchioles" (Seyffert, Lahm), "adenoma pulmonis" (Meyer), "tumor-like malformation" (Meyer, Koboth), "hyperplasia of lung tissue" (Wermbter), "congenital hyperplasia of the lung with deficient development of the alveoli as a result of fetal bronchiectasis" (von Graff, Sternberg), and "adenomatoid overgrowth of the lung" (Koboth). Most authors considered the condition a malformation and not a neoplasm. Lahm thought the cysts arose not only from small bronchioles but also from the infundibula and alveoli.

No one has advanced any definite cause for the condition. Graff spoke of an intrauterine hypertrophy of one lobe (or lung) when he saw that the other lung was just a rudiment. The fact that an entire lobe was malformed indicated to Koboth that the malformation started at a time when the different lobes were

Cases of Congenital Adenomatoid Malformation of the Lung from the Literature

Case	Year	Author	Sex	Age	Position of Lesion	General Anasarca	Hydramnios
1	1897	Stork ¹	M	Newborn	Right lung, middle lobe	Present	
2	1905	von Graff ^{2a}	M	2½ days, a pre mature infant	Left lung, two lobes		
3	1919	Lahm ^{2b}		8 mo intrauterine, stillborn	Left lung, upper lobe	Present	
4	1920	Seyffert ^{2c}	F	8 mo intrauterine, stillborn	Left lung, upper lobe	Present	
5	1923	Sternberg ^{2d}	M	7 mo intrauterine, stillborn	Both lungs	Present	
6	1924	Meyer ^{2e}	F	8 mo intrauterine, stillborn	Right lung	Present	
7	1925	Wermbter ^{2f}	F	Premature (34 cm)	Right lung, two lobes	Present	
8	1926	Nordmann ^{2g}	M	9 mo intrauterine, died after a few breaths at birth	Accessory lung on left side	Present	Present
9	1928	Esch ^{2h}	F	9 mo intrauterine	Right lung, lower lobe	Present	Present
10	1936	Koboth ²ⁱ	F	7 mo intrauterine	Left lung, lower lobe	Present	

already separated in the lung anlage, the absence of arteries in the bronchial walls he explained by the fact that the lung anlage is formed earlier than the intrapulmonary arterial system.

A most remarkable feature of the cases is the constancy of general anasarca (see table), regarded by many as mechanical. Direct compression of the superior and inferior venae cavae was noted by Stork and Koboth. Direct compression of the right auricle was noted by Wermbter and Meyer, while Meyer's tumor also pressed on the upper part of the right ventricle. Nordmann attributed the venous stasis to compression and kinking of the superior and inferior venae cavae as a result of excessive mediastinal and cardiac displacement. Finally Seyffert explained the circulatory embarrassment by excessive positive intra-thoracic pressure (due to rapid expansile growth of the lung) which interfered with the heart in diastole.

Edema of the placenta (Esch, Nordmann and Seyffert) was attributed to systemic venous stasis or cardiac failure.

Pronounced extramedullary hemopoiesis was found in the liver by Esch, Meyer and others and was taken to be compensatory for cardiac failure after an idea sponsored by Schridde,³ but it is more likely an expression of the prematurity of the infants

COMMENT

In our case the pulmonary condition is a malformation because (1) it involves the entire lower lobe of the left lung, (2) it lies within the confines of the left visceral pleura, (3) a branch from the left bronchus leads into the mass, (4) the malformed portion passes directly into normal-looking lung tissue at the periphery without any demarcation or encapsulation and (5) it is an organoid growth consisting of a large number of well differentiated bronchioles lined by columnar cells and surrounded by smooth muscle and a far greater number of terminal bronchioles lined by cuboidal cells with a well formed basement membrane. The essential feature is an excessive overgrowth of the bronchioles, especially of the terminal bronchioles, which causes the marked enlargement of the lobe, while the development of the alveoli is completely suppressed except at the periphery. Thus the mass can be called a hamartoma (tumor-like overgrowth of normal tissue) of the lung except that certain elements, such as cartilage, mucous glands and alveoli, are not present.

In this case, as in most of the reported cases, general anasarca was present, and there is no question that it was of mechanical origin. The lobe was so voluminous that, besides displacing the mediastinum and exerting visible traction on the venae cavae, it must have occasioned a great increase of intrathoracic pressure. This circulatory embarrassment was probably a significant factor in the fetal death in our case and in 4 of the recorded cases. Fetal death can cause premature onset of labor.

Hydramnios, which was present in our case and in 2 recorded cases, seems more than a mere coincidence. Esch's view that it is related to the fetal hydrops is supported by the occurrence of hydramnios in cases of fetal heart disease (DeLee and Greenhill⁴). Hydramnios also induces premature labor.

The presence of both factors causing prematurity in such cases accounts for the fact that in 10 of the 11 recorded cases (including ours) the subject was a premature infant.

The adenomatoid malformation of the lung is a form of, or belongs to the category of, congenital cystic lung, and indeed Koontz⁵ included

3 Schridde, H. *Munchen med Wchnschr* **62** 397, 1910

4 DeLee, J. B., and Greenhill, J. P. *The Principles and Practice of Obstetrics*, ed 8, Philadelphia, W. B. Saunders Company, 1945, pp 39 and 582

5 Koontz, A. R. *Bull Johns Hopkins Hosp* **37** 340, 1925

the case of adenomatoid malformation reported by Stork as an instance of congenital cystic lung. However, cases of adenomatoid malformation of the lung form a special group, as this condition is observed almost exclusively in premature infants, usually with general anasarca and sometimes also with hydramnios, whereas congenital cystic lung is encountered more frequently in adults than in children. Of 374 cases collected by Schenck,⁶ 212 concerned adults, 162, children, and in two thirds of the cases the condition was present after the fifteenth year of age. Anatomically, in the latter disease the cysts are of variable size, may or may not communicate with the bronchi, may be isolated and few or so numerous as to cause a diffuse "honey-combing" of the lung, and may involve one or more lobes of one or both lungs. The fact that coal pigment is not present in the walls of the cysts in adults is generally taken as evidence that the cysts are congenital in contradistinction to bronchiectatic cavities. In the cyst wall, cartilage is generally present but not as constantly as elastic fibers and smooth muscle, and mucous glands are present only in a moderate number of cases. Thus, there is no fundamental anatomic difference between the two conditions. According to Wilson,⁷ the bronchial tree not only continues to grow in size after birth, but continues branching until the end of the seventh year. In this period the tree is still in the formative stage, and therefore malformations can still take place. Excessive size of the cysts or secondary infection determines the onset of symptoms. The patients may die either of asphyxia, due to cystic compression of lung tissue, or of suppuration. The time of onset of the malformation is evidently the deciding factor for the difference of the clinical syndrome, the mode of death and certain anatomic variations. It is remarkable that the prenatal type is so rare (10 cases up to the present) as compared with the postnatal type, which is of relatively common incidence (381 cases up to 1937, according to Schenck). As regards the primary cause of the malformation in both types, nothing definite is known other than that in certain cases the postnatal type is familial (Neisser⁸, Sandoz⁸).

A feature of interest in our case and Koboth's case was the presence of occasional small foci of tubular structures lined by a single layer of very tall columnar epithelium of the type found in the stomach and the colon, which is quite different from the pseudostratified columnar epithelium of the ordinary bronchial system. Since the lung is of endodermal origin, the presence of such tall columnar epithelium of gastrointestinal type is not entirely unexpected. Such foci of tall

6 Schenck, S. G. *Am J Roentgenol* **35** 604, 1936

7 Wilson, G. H. *Am J Anat* **41** 97, 1928

8 Cited by Schenck⁶

columnar cells probably gave rise to the so-called enterocystoma of the lung (Ewing⁹), viz, small or large cysts lined by colonic epithelium inside the lung as instances of dysontogenesis. In such cases the lung tissue surrounding the cyst or cysts is quite normal.

The observation that foci of colonic epithelium are present in adenomatoid malformation of the lung brings up for discussion another condition described under the names of "adenomatosis of the lung" (Richardson¹⁰), "pulmonary adenomatosis" (Sims¹¹, Simons¹²) and "pulmonary mucous epithelial hyperplasia" (Bell¹³, Taft and Nickerson¹⁴). In this condition, tumor-like nodules or masses are found in the lung and each nodule consists of glandular structures lined by tall columnar epithelium. Adenomatosis of the lung is therefore quite different from adenomatoid malformation of the lung, since the latter is a diffuse malformation without tumor-like nodules and tall columnar epithelial cells are found only in occasional small foci and do not show massive overgrowth. In passing it may be stated that conditions resembling adenomatosis but revealing the character of a true cancer in giving rise to metastases in bronchial and prevertebral lymph nodes (Briese¹⁵) and in bones and regional lymph nodes (Oberndorfer¹⁶) have been described. Furthermore, lesions resembling adenomatosis of the lung may be produced in certain infections in animals, viz, jagziekte of sheep in South Africa and Iceland and progressive pneumonia of sheep and horses in Montana (Dungal¹⁷, Bonne¹⁸, Wood¹⁹, Norris²⁰), and, strange enough, even in this type of hyperplasia of tissues, on an infectious basis, Aynaud²¹ has found metastasis to the hilar lymph nodes. In this infectious type and in human adenomatosis of the lung, the tall columnar epithelial cells arise, it is believed, from the bronchiolar and alveolar epithelium by metaplasia as a reaction to chronic irritation, rather than from embryonic cell rests.

9 Ewing, J. *Neoplastic Diseases*, ed 4, Philadelphia, W. B. Saunders Company, 1940, p. 1065.

10 Richardson, G. O. *J. Path. & Bact.* **51**: 297, 1940.

11 Sims, J. L. *Arch. Int. Med.* **71**: 403, 1943.

12 Simons, M. A. *Am. J. Path.* **23**: 413, 1947.

13 Bell, E. T. *Am. J. Path.* **19**: 901, 1943.

14 Taft, E. B., and Nickerson, D. A. *Am. J. Path.* **20**: 395, 1944.

15 Briese, cited by Bonne¹⁸.

16 Oberndorfer, S. *Virchows Arch. f. path. Anat.* **275**: 728, 1930.

17 Dungal, N. *Proc. Roy. Soc. Med.* **31**: 497, 1938.

18 Bonne, C. *Am. J. Cancer* **35**: 491, 1939.

19 Wood, D. A., and Pierson, P. H. *Am. Rev. Tuberc.* **51**: 205, 1945.

20 Norris, R. F. *Arch. Path.* **43**: 553, 1947.

21 Aynaud, cited by Sims¹¹ and by Dungal¹⁷.

SUMMARY

A case of congenital adenomatoid malformation of one lobe of a lung is reported. Only 10 similar cases have been described in the literature. The mechanical circulatory embarrassment may cause death of the fetus. Hydramnios was present in 3 of the 11 cases and is possibly related also to the fetal hydrops.

The condition is of the nature of a malformation which starts when the lung anlage has already undergone lobulation but cartilage, arteries, mucous glands and alveoli are not yet differentiated. The lesion is more than a simple malformation, however, since there is an overgrowth of the bronchioles—a hamartoma, which is responsible for the enlargement.

Congenital adenomatoid malformation of the lung belongs to the general category of congenital cystic lung, but forms a special subgroup by itself.

BEHAVIOR OF HODGKIN'S DISEASE NODES TRANSPLANTED INTO THE ANTERIOR CHAMBER OF THE RAT'S EYE

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THIS study was undertaken in order to observe the behavior of Hodgkin's disease tissue and the reaction induced by it after it had been transplanted to the anterior chamber of the rat's eye.

The method of study adopted was that of Greene,¹ who has utilized the anterior chamber of the eye for transfers of homologous and heterologous embryonic and neoplastic tissue.

Although it was known to us (by personal communication) that Greene had failed to obtain any "takes" in some 20 Hodgkin's tissue transplants, repetition of this work was considered justified for a number of reasons:

1 We wished to determine whether failure of transplantation could be correlated with the various types of histologic pattern encountered in this disease.

2 We wished to study the early changes in the transplant and the iris reaction of the host, comparing these with control material. The iris reaction was to be followed to see whether a specific inflammatory reaction occurred, and thus secure a clue to the presence of a possible etiologic agent.

3 Since tissue culture studies of Hodgkin's disease nodes were already in progress, it seemed advisable to extend the experiment to include parallel transplantation of both fresh tissue and fragments which had been maintained in tissue culture. We hoped to determine whether a period of *in vitro* cultivation would alter the biologic properties of the tissue as far as transplantability is concerned.

MATERIALS AND METHODS

White rats ranging from 2 to 4 months of age were used as hosts for the majority of the transplants. Rats were chosen mainly because an active colony was

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1 Greene, H. S. N. J. Exper. Med. **73**: 461, 1941.

available and also because it seemed pointless to use guinea pigs, since they had already been shown to be of no value for Hodgkin's disease transplants. Mice proved to be unsuitable because of technical difficulty in effecting the transfer of the tissue without producing traumatic cataracts. A few rabbits were used, but since the results were negative, we concentrated on the rats.

The number of rats used for each experiment varied from 3 to 19. Most of them were killed at intervals of five to ten days for microscopic examination of the transplant and the eye tissues, a few were retained for longer periods, up to six months.

The transplantation technic was that of Greene, who gave us personal instruction in this technic.

The tissues used were, with one exception, of human origin. For the main portion of the experiment we used lymph nodes removed at operation from 27 patients. Portions of full term placenta were also transferred as an example of presumably inert tissue for comparison. Rat embryo tissues were used as a control of the technic and, finally, we injected tissue culture medium alone as a control for the cultured fragments.

Thirteen of the lymph nodes showed Hodgkin's disease. The remainder of the nodes manifested various lesions, including lymphoid hyperplasia (2 nodes), tuberculous lymphadenitis (1), reticulum cell sarcoma (3), lymphosarcoma (2) and metastatic carcinoma (6).

Of the 13 Hodgkin's disease transplants, 7 were fresh tissue and 11 were tissue culture fragments, double transfers of both fresh and cultured tissue were carried out in 5 instances. The various lymphoid controls were evenly divided between fresh and cultured fragments.

The Hodgkin's disease lymph nodes presented varying histologic patterns. According to the classification of Jackson and Parker,² 2 of these were paragonuloma, 10 were granuloma and 1 was Hodgkin's sarcoma. Four of the nodes were markedly fibrotic.

The cultures were grown by Miss Augusta Hollender, in Carrel flasks, type D-5, in a medium consisting of a mixture of chick plasma, chick embryo extract, Tyrode's solution and human umbilical cord serum in the solid phase, and chick extract, human cord serum and Tyrode's solution in the fluid phase. From 20 to 25 fragments, each about 1 cu mm, were placed in each flask. Since it was impossible to dissociate the tissue culture fragments from the tenacious medium, the latter was also introduced into the animal's eye along with the tissue. Control studies subsequently proved that the medium was bland.

The cultures were in most instances forty-eight to seventy-two hours old at the time of transplantation, the time being selected according to the richness of the outgrowth and the number and the type of cells in the zone of migration, both of these phenomena being quite variable. In 2 cases we used six week cultures composed entirely of sheets of fibroblasts. One of these was from a node showing Hodgkin's disease, the other, from one showing lymphoid hyperplasia. The control tissue culture material, other than the six week culture of a hyperplastic node consisted of cultures of tissues presenting metastatic carcinoma of the thyroid gland, tuberculous lymphadenitis, lymphoid hyperplasia, reticulum cell

2 Jackson, H, Jr, and Parker, F, Jr. *Hodgkin's Disease and Allied Disorders*, New York, Oxford University Press, 1947.

sarcoma (2 cases) and lymphosarcoma. All these had been maintained for forty-eight to seventy-two hours in Carrel flasks before portions were transplanted to the eye.

Fate of Transplanted Fragments—Regardless of the inherent lesion of the transplanted lymphoid tissue, in the eye the behavior of the transplant followed a set pattern.

During the first week the fragment was grossly observed to be surrounded by a filmy halo. Microscopic sections showed this to be a clear coagulum. In some instances the fragment slowly disintegrated and eventually disappeared in the first ten to twenty days. In others it became dense and white, with irregular edges, retaining in general the shape of the original fragment but decreasing slightly in size. If the fragment was still visible after two to three weeks, it usually remained unchanged until the animal was killed, even up to six months after transplantation.

Microscopic examination at five to ten day intervals showed that, with the exception of a few lymphocytes, the loose cellular constituents of the transplant disappeared during the first week, leaving only a small fibrous mass, the center of which in many instances showed degeneration and necrosis. Those fragments which grossly appeared dense and white after thirty or more days in the anterior chambers were composed of acellular, avascular fibrous tissue.

Sternberg-Reed cells were not observed in any of the Hodgkin's disease fragments even when they were examined by serial section as early as three days after transplantation.

All control tissues, both fresh and cultured, showed essentially the same changes as those described for Hodgkin's disease tissue. No "takes" were obtained. Those fragments which did not undergo early necrosis and autolysis eventually became fibrotic.

Iris Reaction—In all instances the transplanted fragment adhered to the iris at one point or another, and there was usually a marked lymphocytic reaction in the iris along the line of attachment. Rarely eosinophilic leukocytes infiltrated the site of attachment, usually to a slight degree. After the first week, giant cells with numerous peripheral nuclei were frequently observed both in the iris at the point of attachment and in the periphery of the transplant itself, surrounding the central necrotic zone.

The lymphocytic reaction of the iris was most intense in those animals which were killed during the first week and in which the transplant showed extensive necrosis. Fibrotic fragments were usually accompanied by only a slight reaction of the iris.

No Sternberg-Reed cells were ever observed in serial sections through the eye.

Essentially the same iris reaction was seen with the fresh tissue and with the cultured controls, regardless of the inherent pathologic process in the tissue before transplantation. The same changes were noted around a fragment of normal human placenta as around fragments of reticulum cell sarcoma and tissues showing varying histologic types of Hodgkin's disease.

The tissue culture medium alone produced no iris reaction.

COMMENT

Like the guinea pig used by Greene, the rat proved to be a poor host for lymphoma planted in the anterior chamber of the eye.

Our lack of success with the transplantation of cancer tissues used for control purposes may be largely due, again, to the fact that the rat is a poor host for heterologous transplants. Homologous transplants of rat embryo grew well, almost filling the chamber in two weeks. This demonstrated to us that the failures with heterologous tissue were not due to error in the technic of transfer.

Tissue grown first *in vitro* and later planted in the anterior chamber fared no better than fresh tissue.

The reaction of the host rat to all heterologous tissue planted in the eye was of a chronic inflammatory nature and nonspecific in type. We failed to find anything which could be interpreted as conforming to a Hodgkin's disease pattern.

HYPERTENSIVE CARDIOVASCULAR DISEASE

An Experimental Study of Tissue Changes in Bilaterally Nephrectomized Dogs

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THAT the kidney plays an important role in the genesis of hypertensive cardiovascular disease ("malignant hypertension") is generally accepted, but the mechanism involved is still a matter of controversy. Thus many¹ believe that this disease results from the action of a pressor substance produced by the ischemic or otherwise disordered kidney, others² maintain that some renalcretory function is in abeyance. Others look on the disease as an ill defined reaction to stress related to such diverse clinical states as rheumatic fever or periarteritis nodosa.³ Bilateral nephrectomy affords an obvious procedure for elucidating the role of the kidney in the genesis of hypertensive cardiovascular disease and has been performed by numerous investigators. Since this operation has not resulted consistently in the development of the two major characteristics of the disease (an elevated blood pressure and arteriolar lesions), previous workers have concluded that the presence of altered renal tissue is necessary for the development of this disorder.⁴ However, in none

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1 Goldblatt, H, in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1938, p 237

2 Grollman, A, in Pincus, G. Recent Progress in Hormone Research, New York, Academic Press, Inc, 1947, vol 1, p 371

3 Selye, H. Ann Int Med **29** 403, 1948

4 (a) Goldblatt, H. Ann Int Med **11** 69, 1937 (b) Winternitz, M C, Mylon, E, Waters, L L, and Katzenstein, R. Yale J Biol & Med **12** 623, 1940 (c) Cash, J R. Proc Soc Exper Biol & Med **23** 609, 1926 (d) Hartwich, A. Ztschr f d ges exper Med **69** 462, 1930 (e) Harrison, T R, Mason, M F, Resnik, H, and Rainey, J. Tr A Am Physicians **51** 280, 1936 (f) Katz, L N, and others. Am Heart J **17** 334, 1939 (g) Braun-Menendez, E, and others. Hipertension arterial nefrogene, Buenos Aires, El Ateneo, 1943

of their studies was it possible to prevent the relatively early effects of the uremic state, and one could, therefore, always attribute the observed negative results to the poor state of the animals and their short period of survival following nephrectomy. As a matter of fact, in some of these earlier experiments occasional animals that did not deteriorate too rapidly displayed moderate to prominent elevations of blood pressure.⁵ These isolated examples of hypertensive vascular disease following bilateral nephrectomy, being exceptional rather than the rule, were disregarded in the ultimate conclusions drawn from these experiments.

In the present study we have reinvestigated the effects of nephrectomy on the blood pressure and the pathologic changes induced by this operation. By prolonging the life of our animals and concomitantly improving their general state of health, it has been possible to obtain consistent results and to obviate the objections raised against previous work in this field. The prolongation of the life of the nephrectomized dog was made possible by the use of electrolyte-free diets and the application of an artificial kidney, the use of which has been reported elsewhere.⁶ By these procedures, it has been demonstrated that in the dog bilateral nephrectomy is followed consistently by an elevation of blood pressure, which frequently reaches high levels.⁷ In the present communication we wish to present results dealing with the other accepted component of hypertensive cardiovascular disease, viz., the morphologic changes occurring throughout the body.

The most informative of the extant studies on the effects of bilateral nephrectomy in dogs is that of Winternitz, Mylon, Waters and Katzenstein,⁸ 1 of whose animals survived ten days, while 6 survived on the average slightly over seven days. Although in these 6 dogs there was a tendency for the blood pressure to rise, in 1 instance reaching levels as high as 200 mm of mercury (mean pressure), these authors disregarded these rises and concluded "that the kidney is not primarily concerned in the maintenance of the normal blood pressure." The blood nonprotein nitrogen of their animals, the acidosis and the hypochloremia reached significant proportions, but at autopsy these workers encountered no specific gross lesions. Occasional sub-endocardial, valvular and visceral hemorrhages and focal necrosis involving heart muscle and the smooth muscle of the arterial wall

⁵ Winternitz and others^{4b} Harrison and others^{4c}

⁶ Vanatta, J., Muirhead, E. E., and Grollman, A. *Am J Physiol* **156** 443, 1949

⁷ Grollman, A., Muirhead, E. E., and Vanatta, J. *Am J Physiol* **157** 21, 1949

⁸ Winternitz, M. C., Mylon, E., and Katzenstein, R. *Yale J Biol & Med* **13** 595, 1941, footnote^{4b}

and of the alimentary and genitourinary tracts were observed after careful search but only in minimal degrees. Ligation of both renal arteries or of both ureters, on the other hand, was found to be associated with great accentuation of these lesions. If crude kidney extracts were injected after bilateral nephrectomy, there was accentuation of these lesions and of the elevation of blood pressure. Winternitz and associates concluded, therefore, that renal extracts contained pressor, necrotizing and hemorrhagic factors which presumably were the responsible agents in the causation of "malignant hypertension" as suggested by Goldblatt¹. Winternitz and associates, however, described various morphologic changes in the body with greater thoroughness and attempted to separate pressor and lesion-inducing factors present in renal extracts. These authors realized that material absorbed from necrotic kidneys could not be the only factor responsible for the observed lesions, since similar, although minimal, changes occurred following bilateral nephrectomy alone.

The observations and interpretations of Winternitz and associates are emphasized here because our observations of the morphologic changes induced in dogs by bilateral nephrectomy are consistent with their earlier work, although the interpretations given these findings differ widely. The widespread fulminant lesions described by Winternitz and co-workers as following ligation of the ureters and the injection of renal extracts in nephrectomized animals have been observed by us following bilateral nephrectomy alone. Moreover, as mentioned in a foregoing paragraph, such animals under our experimental conditions consistently displayed after nephrectomy a rising blood pressure which attained significant proportions by the seventh to tenth day. Thus, under the proper conditions a hypertensive vascular disease does follow bilateral nephrectomy and is accompanied by the lesions characteristic of "malignant hypertension."

The present observations are based on the gross and microscopic changes occurring in two groups of 71 dogs after bilateral nephrectomy. The first group, consisting of 27 animals, received electrolyte-free food but were allowed to die in uremia without being attached to the artificial kidney. In the second group, of 44 dogs, survival and improved body state were extended by the use of an electrolyte-free diet plus periodic application of the artificial kidney. The former group serves as a control so far as any effects that might be induced by the artificial kidney are concerned.

In addition to observing dogs, we have examined the tissues of patients coming to autopsy to correlate the experimental findings with those observed in the human subjects. To demonstrate the identity of the experimental and clinical findings, we have included the study of the human tissues in the present report.

METHODS

Adult mongrel dogs were used throughout. The technics used in performing the nephrectomies and in the application of the artificial kidney are described elsewhere.⁹ By a modification of the procedure of Kolff and Noordwijk,¹⁰ *in vivo* dialysis was conducted for three hours at intervals of 3 to 5 days. At the end of the dialysis, if there was severe anemia, transfusions of concentrated red blood cells (properly cross matched) were given. Electrolyte-free food and water were offered the animals *ad libitum*.

Promptly after the death of the animals, autopsies were made, and the tissues were fixed in 4 per cent formaldehyde solution. These were prepared in the usual manner and stained with hematoxylin and eosin. On a few occasions Masson's trichrome stain as modified by Goldner¹¹ was also utilized. The tissues were selected from areas showing pathologic changes grossly or, when no such changes were evident, were taken by multiple random sampling.

Several different types of controls were available. Any effects induced by the artificial kidney were controlled as mentioned in a foregoing paragraph. The ablated kidneys acted as specific controls for each animal. In addition, tissues were taken from the following group of normal dogs after the stated manipulations and compared with the tissues from the nephrectomized animals: (1) animals used as blood donors, (2) animals subjected to *in vivo* dialysis with the artificial kidney, (3) animals whose tissues were allowed to autolyze prior to fixation, (4) animals receiving large doses of heparin, and (5) animals subjected to various types of oligemic shock.¹²

Mean blood pressures were obtained by direct puncture of the femoral artery and a mercury manometer.¹³

RESULTS

Blood Pressure—Although the specific blood pressure effects of bilateral nephrectomy and other renal manipulations are discussed in detail elsewhere,⁷ the scatter graph of figure 1 is given here to indicate the tendency of blood pressures to rise after nephrectomy in animals not aided with the artificial kidney (average survival, 47 days), as well as in those in which survival was prolonged (average survival, 71 days) by the use of the artificial kidney.

Survival—The survival period of all the animals varied between 3 and 195 days. The 195 day survival constitutes the longest survival of a bilaterally nephrectomized dog with which we are familiar.

The survival period may be analyzed by separating the dogs into three groups, which are listed in frequency distribution style in table 1. The first group consists of 27 animals that died without being attached to the artificial kidney. In this group the average survival in the 20 instances in which the exact period of survival was known amounted to 47 days (113 hours). Sixty-five per cent of this group survived between 5 and 6+ days. The second group consists of 31 animals

9 Vanatta and others⁶ Grollman and others⁷

10 Kolff, W. J., and Noordwijk, J. *The Artificial Kidney*, Kampen, Holland, J. H. Kok, 1946

11 Goldner, J. *Am J Path* **14** 237, 1938

12 (a) Muirhead, E. E., Kregel, L. A., and Hill, J. M. *Arch Surg* **47** 258, 1943. (b) Muirhead, E. E., and Hill, J. M. *J Lab & Clin Med* **29** 239, 1944

13 Grollman, A. *Am J Physiol* **147** 647, 1946

succumbing after one application of the artificial kidney. In this group the average survival extended to 62 days (149 hours), and 70 per cent of this group survived for 6 to 8 days or longer. The third group, of 11 animals, were aided with the artificial kidney on 2 occasions and survived between 8 and 9+ days (average, 90 days or 216 hours).

Although the use of an "artificial kidney" and an "electrolyte-free" diet extends the survival of bilaterally nephrectomized animals, in view of the lesions to be described next it is not surprising that survival is not prolonged indefinitely. The generalized degenerative effects, particularly those involving the heart and the blood vessels, apparently do not allow for an indefinite survival.

Gross Lesions—The gross changes that were present in each instance consisted of generalized hyperemia of the viscera, pulmonary edema and cardiac

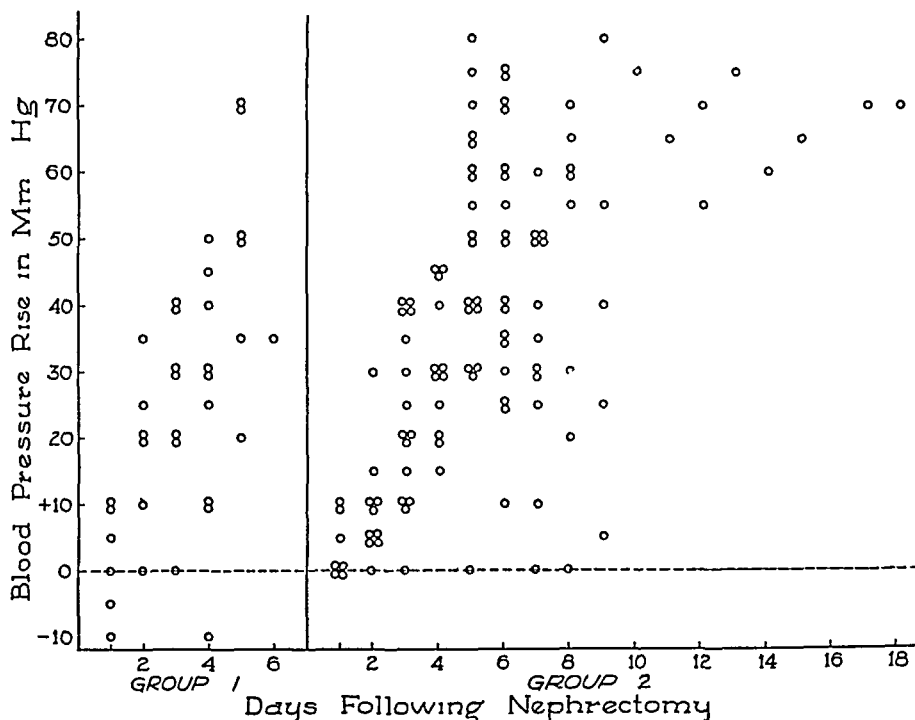


Fig 1—A scatter graph of the blood pressures observed following bilateral nephrectomy in dogs. At the left are readings on dogs not subjected to dialysis (group 1), at the right, data on animals treated by dialysis with the artificial kidney (group 2).

dilatation, more prominent on the right side. The next most commonly encountered change consisted of hemorrhages. In the heart these were found most consistently in the subendocardial tissue of both the right and the left ventricle but preponderantly in the latter. They usually involved the papillary muscles, the apex and the intraventricular septum from its midportion down toward the apex. At times, intramural myocardial hemorrhages were evident grossly. Pulmonary hemorrhages were likewise common. These involved the parenchyma but were evident from the pleural surfaces. The hemorrhagic areas were usually round and measured 2 to 5 mm in diameter. They were scattered throughout all lobes but were more frequent in the lower lobes. Occasionally, large pulmonary hemorrhages were encountered, less commonly, confluent hemorrhages gave rise to focal or

lobar hemorrhagic consolidation. Pulmonary infarcts were rarely encountered. Next in frequency were hemorrhages of the gastrointestinal tract, which usually involved the entire tract from stomach to rectum. In the stomach the hemorrhages were found mostly in the mucosa and submucosa and at times extended into the lumen. Rarely an animal seemed to succumb to a massive gastric hemorrhage. The hemorrhages of the intestine were usually in the muscularis and serosa. Again in rare instances massive intestinal hemorrhage occurred. Intussusception of the intestine was observed to occur in 11 per cent of the entire group, with over 60 per cent of the intussusceptions occurring after the seventh day. These were associated with hemorrhagic infarction, obstruction and hemorrhages into the bowel.

Microscopic Lesions—Microscopically, certain consistent alterations, such as changes associated with circulatory disturbances and usually ascribed to congestive heart failure, were observed in the entire group. Generalized visceral venocapillary hyperemia was prominent and affected heart, lungs, abdominal viscera and brain. In addition, the heart at times displayed interstitial edema.

TABLE 1—*The Survival of Bilaterally Nephrectomized Dogs With and Without the Application of the Artificial Kidney*

Group 1 Not Subjected to Artificial Kidney		Group 2 Subjected to Artificial Kidney Once		Group 3 Subjected to Artificial Kidney Twice	
Dogs	Period of Survival, Days	Dogs	Period of Survival, Days	Dogs	Period of Survival, Days
2	3 to 3+	3	4 to 4+	4	8 to 8+
5	4 to 4+	6	5 to 5+	7	9 to 9+
10	5 to 5+	9	6 to 6+		
3	6 to 6+	11	7 to 7+		
		2	8 to 8+		
Average	4.7		6.2		9.0

The lungs were consistently the seat of hyperemia, edema and focal capillary hemorrhages. Eosinophilic emboli were observed within the small vessels of the lungs, associated with certain of the pulmonary hemorrhages, in 16 dogs, 2 of which were in group 1 (no in vivo dialysis). In structure these embolic masses were lumpy and granular and resembled the smudged media of the arteries to be described. At times they contained entrapped nuclear remnants, suggesting a causal relationship to the smudged vessels, and 12 of these animals, 10 of which survived 7 days or longer, had prominent smudging of arterial media elsewhere in the body. The pulmonary edema on each occasion involved the dependent areas and at times was severe and generalized. In the latter instances, the bronchi and the trachea were filled with a frothy pink fluid. Focal atelectasis occurred, usually in the presence of more prominent hemorrhages.¹⁴ The hyperemia of the liver was associated with centrilobular damage, usually in the form of central atrophy but in more severe cases in the form of central fatty metamorphosis or outright central necrosis. Not infrequently, the adrenal cortex was found to be prominently infiltrated with polymorphonuclear neutrophilic leukocytes. (This finding has been observed frequently following fatal oligemic shock due to a severe

¹⁴ A small percentage of the dogs had chronic interstitial pneumonitis. This change apparently constituted a previously existent state and did not seem to alter the findings except in the lungs.

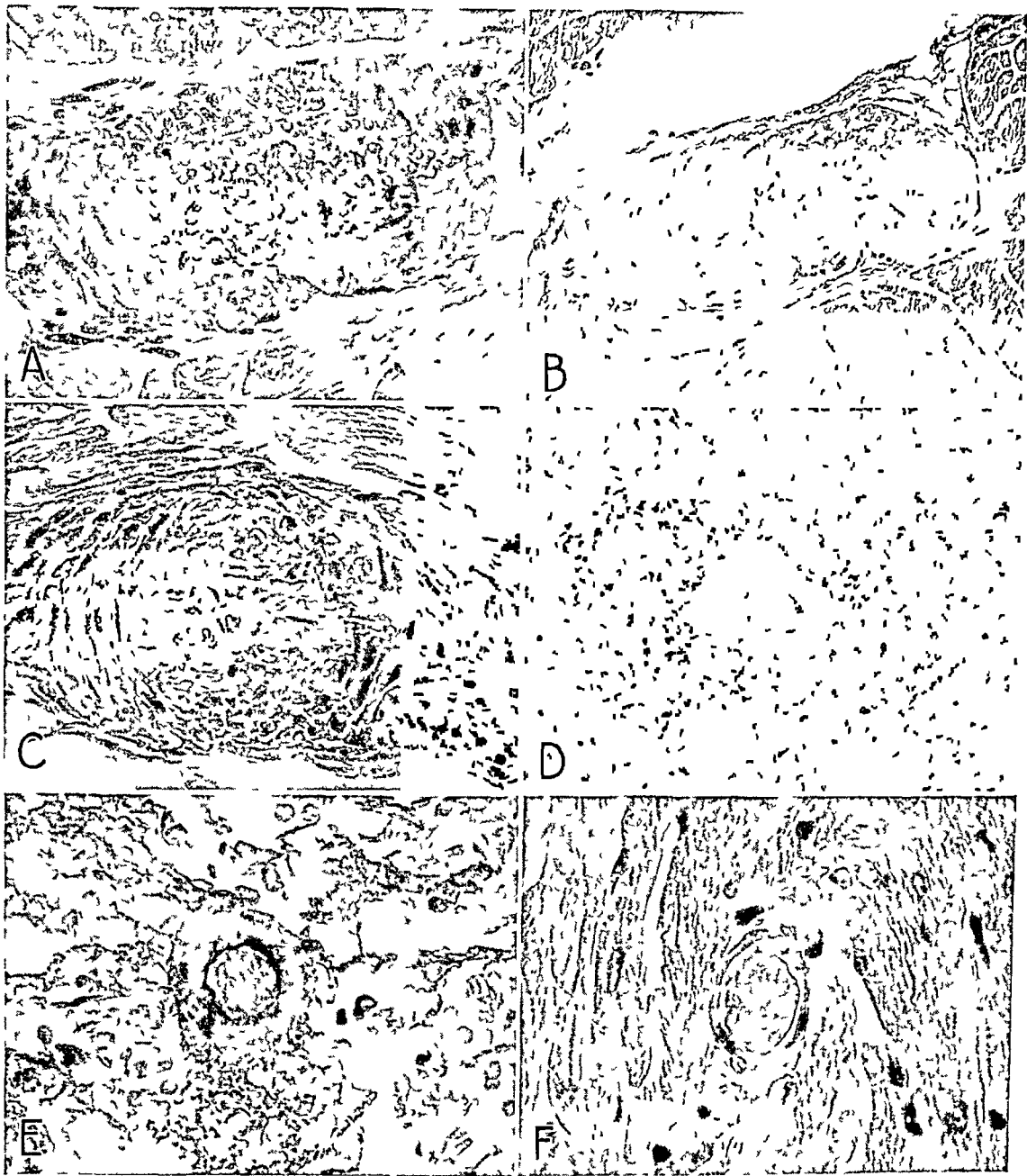


Fig 2—The vascular lesions, in various stages of development, as observed in nephrectomized dogs with hypertensive cardiovascular disease. *A* (dog 28, survival, 5 days, blood pressure, 150, no dialysis), small artery of the myocardium showing degeneration and necrosis of the smooth muscle fibers of the media. Notice the hyaline swelling of fibers, absence of nuclei focally with early lumpy granular change of the sarcoplasm, and the hyaline swelling of other fibers with pyknotic nuclei. The individual cell boundaries are maintained by most fibers, although early smudging can be seen in the left lower area. $\times 211.5$

B (dog 75, survival, $7\frac{1}{4}$ days, blood pressure, 135, subjected to one dialysis), small artery of the myocardium showing degeneration and necrosis of the media more advanced than in *A*. Most fibers have lost their nuclei and

(Legend continued on next page)

freeze^{12a} and following fatal burns¹⁵ The observation, therefore, does not seem to have a specific connotation) The brain usually displayed microscopic "collar" hemorrhages into the Virchow-Robin spaces

Aside from the circulatory disturbances that can be considered as due mainly to congestive heart failure, there were specific microscopic changes at various sites Certain of these morphologic changes are considered by workers in this field as a necessary component of so-called malignant hypertension, namely, the arterial and arteriolar necrosis or the "necrotizing arteriolitis" of Fahr¹⁶ In its ultimate state this lesion consisted of necrosis of the media with an eosinophilic smudging of the vessel wall and had all of the features usually ascribed to it, including in addition to the smudging (1) focal or segmental involvement of a given vessel, (2) partial or complete circumferential involvement, (3) intact endothelium without thrombosis, (4) rupture of an occasional vessel with hemorrhage, (5) spreading of the eosinophilic substance into the adventitial and periadventitial areas, (6) narrowing of the lumen of smaller vessels and (7), in isolated instances, spreading of the eosinophilic substance into the lumen On very rare occasions the adventitia of a smudged artery contained a few scattered round cells of the lymphocytic series Polymorphonuclear leukocytes, eosinophilic granulocytes, fibrinoid degeneration and other features of periarteritis nodosa were not observed Moreover, in these same animals other arteries failed to display this peculiarity Klemperer and Otani¹⁷ noted the same finding in malignant hypertensive vascular disease of human beings

It is believed that the mechanism of this arterial and arteriolar necrosis can be defined better on the basis of the findings in other vessels with or without slight smudging There appeared to be alterations of the vessel wall which might be considered as precursors of the ultimate smudging effect and which constituted a part of the generalized smooth muscle effect to be discussed subsequently The smooth muscle fibers of the media not infrequently revealed a swelling of the

15 Dunphy, J E, and Gibson, J G Surg, Gynec & Obst **72** 832, 1941

16 Fahr, T Virchows Arch f path Anat **226** 119, 1919

17 Klemper, P and Otani, S Arch Path **11** 60, 1931

display hyaline swelling with retention of cell outlines The endothelium is intact, and the luminal blood is not clotted $\times 166.5$

C (dog 26, survival, 4 days, blood pressure, 160, no dialysis), small artery of skeletal muscle The inner half of the media around the circumference displays necrosis of the smooth muscle fibers The outer half shows hyaline swelling of sarcoplasm and pyknosis of nuclei There appears to be a tendency for the inner necrotic portion to slough into the lumen $\times 211.5$

D (dog 40, survival, $9\frac{1}{4}$ days, blood pressure 200, 2 dialyses), small artery of the submucosa of the stomach showing complete necrotic smudging of the media The endothelium is intact and the luminal blood is not clotted The eosinophilic smudge has spread into the adventitia Scattered cells of the lymphocytic series are seen in the adventitia The adjoining connective tissue is edematous and has been the seat of a recent hemorrhage $\times 166.5$

E (dog 40, survival, $9\frac{1}{4}$ days, blood pressure, 200, 2 dialyses), arteriole of the submucosa of the stomach showing necrosis of media with hyaline smudging The lumen contains some of the smudged material, and the surrounding connective tissue is edematous and hemorrhagic $\times 294$

F (dog 14, survival, $8\frac{1}{4}$ days, blood pressure, 160, 2 dialyses), capillary of a viable area of the myocardium, occluded and distended by eosinophilic smudged material The endothelial wall is intact Such structures suggest sloughing of necrotic media into the lumen of an artery or an arteriole, with plugging of adjacent capillaries $\times 750$

sarcoplasm, with the usual fine and delicate longitudinally striated appearance (the change ascribed to myofibrils) giving way to a diffuse, dense and hyaline appearance. The fibers still retained their close apposition and remained as individual units, but the sarcoplasm was obviously altered. Associated with this cytoplasmic change was a prominent degree of pyknosis of the nuclei. From this change there appeared to be a transition into karyorrhexis or karyolysis of the nuclei and a fusion of the hyaline material into the eosinophilic smudge. Obviously, where the smudging had already occurred, one could not be certain that these seemingly preliminary changes had preceded it, but concomitant existence of the two changes was so common as to constitute strong collateral evidence for this view.

The arterial and arteriolar lesions involved mainly the "intimate vasculature" of the viscera. The two most common sites were the heart and the gastrointestinal tract. Much less frequently the lesion was encountered in the periadrenal connective tissue, the liver, the esophagus and the omentum. Only twice was the ultimate smudging change seen in random sections of skeletal muscle, and only once was it observed to involve the meningeal vessels.

Usually partially or completely smudged vessels retained their endothelial lining. On a few occasions, however, there appeared to be spilling of the eosinophilic substance into the lumen with partial or complete blockage. In certain cases it was possible to observe a capillary with intact wall and a plug of this substance within the lumen, forming a cast. No instance of classic thrombosis (thrombocytic column, fibrin, red and white blood cells) was observed in the entire series.

Eleven dogs displayed focal necrosis of the gastric mucosa and submucosa. This was usually associated with necrosis of submucosal arterioles, hemorrhages and collections of polymorphonuclear neutrophilic leukocytes. Focal necrosis of similar type was less commonly observed in the intestinal mucosa, the adrenal glands, the pancreas and the liver. In some cases hemorrhages were observed in the superficial zones of the gastric mucosa without the demonstration of arteriolar necrosis. Here there appeared to be disintegration of superficial capillaries.

The other main specific alteration involved the muscle tissue elsewhere in the body, particularly the myocardium. The changes were observed to involve isolated segments of individual muscle fibers, small groups of fibers and at times relatively large areas, as the greater portion of a papillary muscle. The earlier phases of this change were evident where an isolated segment of an otherwise intact fiber was affected. Here the sarcoplasm lost its cross striations and became transformed into a lumpy granular eosinophilic mass. These lumpy areas frequently became arranged transversely and alternated with clearer zones which had a moth-eaten appearance. Where nuclei remained in these areas, they were pyknotic and shriveled, but the wall of the fiber remained intact. When several adjoining fibers were involved, at times the walls were intact and at other times there was fusion of the eosinophilic substance. Under such circumstances hemorrhages also occurred. The larger areas of involvement showed similar changes but to a more advanced degree. Some fibers retained their sarcolemma while others coalesced. Hemorrhages were prominent and, in addition, polymorphonuclear neutrophilic leukocytes infiltrated the area to a prominent degree. At no time was suppuration observed. In some instances smudged blood vessels were prominent in these zones but in others a close search and additional sections through the area failed to reveal their presence. Another modification of the same process was seen in areas where no fiber remnants could be identified and only microscopic pools of

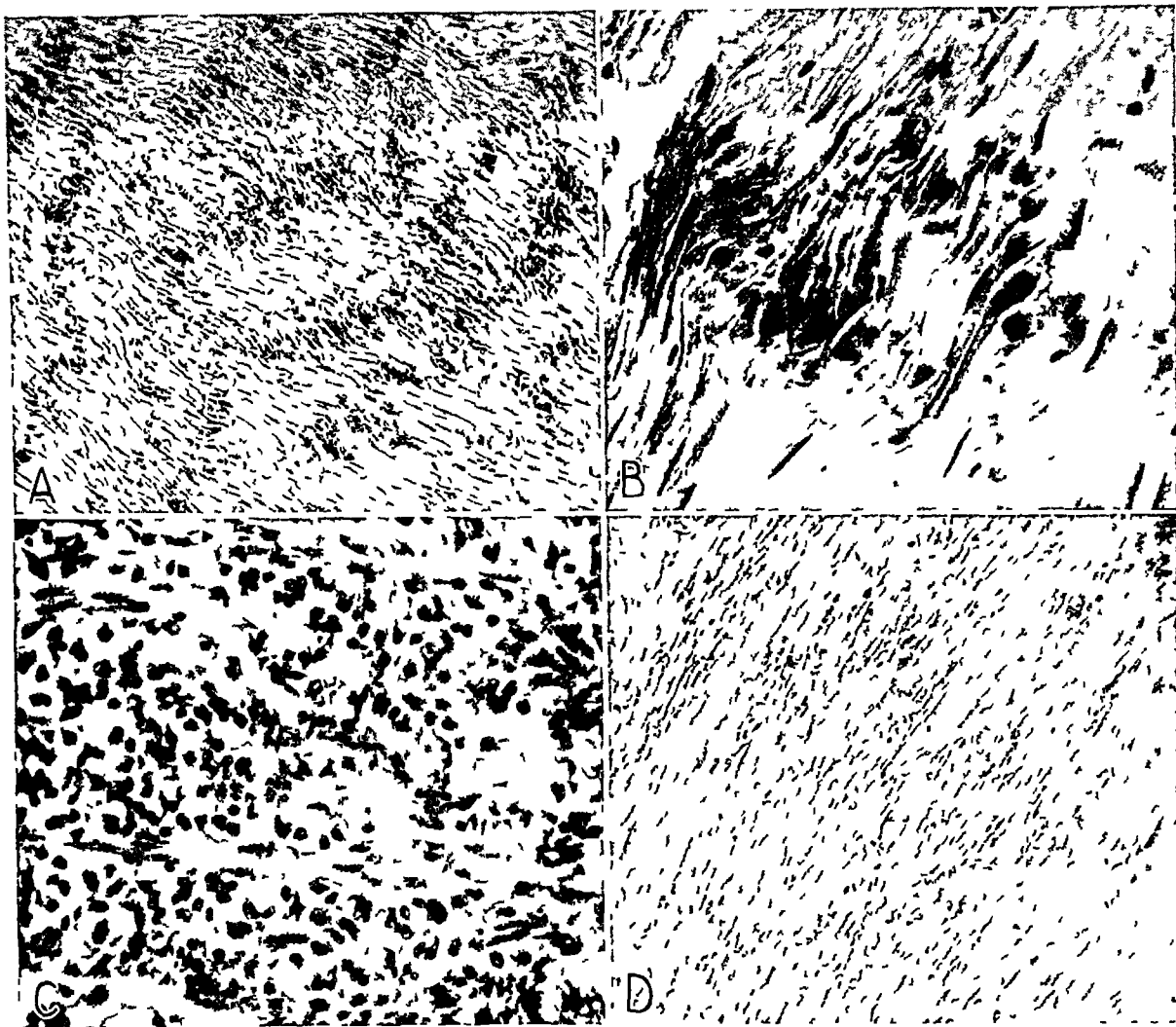


Fig 3—Lesions of smooth muscle as observed in dogs in which hypertensive cardiovascular disease developed following bilateral nephrectomy. *A* (dog 21, survival, 5 days, blood pressure, 140, no dialysis), circular layer of the muscular coat of the small intestine showing irregular transverse bands (palisades) of degenerated smooth muscle fibers. The fibers display individual hyaline swelling of the sarcoplasm and pyknosis of the nuclei. Between these zones the fibers are of near normal thickness. Such bands have suggested foci of contraction and degeneration $\times 50$.

B (dog 24, survival, 6¼ days, blood pressure, 170, no dialysis), circular layer of the muscular coat of the small intestine showing a focus of smooth muscle degeneration and necrosis. The fibers are markedly swollen and hyaline. In some the nuclei are pyknotic, in others they are absent $\times 199$.

C (dog 42, survival, 4 days, blood pressure, 140, no dialysis), mucosal villus of the small intestine showing hyaline swelling and pyknosis of smooth muscle fibers. The sarcoplasm has undergone hyaline change in focal areas, giving the fibers a bulbous appearance $\times 199$.

D (dog 79, survival, 9¼ days, blood pressure, 190, 2 dialyses), necrosis of smooth muscle of the circular coat of the large intestine. Hyaline swelling and lumpy granular changes are seen. Neutrophils have infiltrated this area $\times 605$.

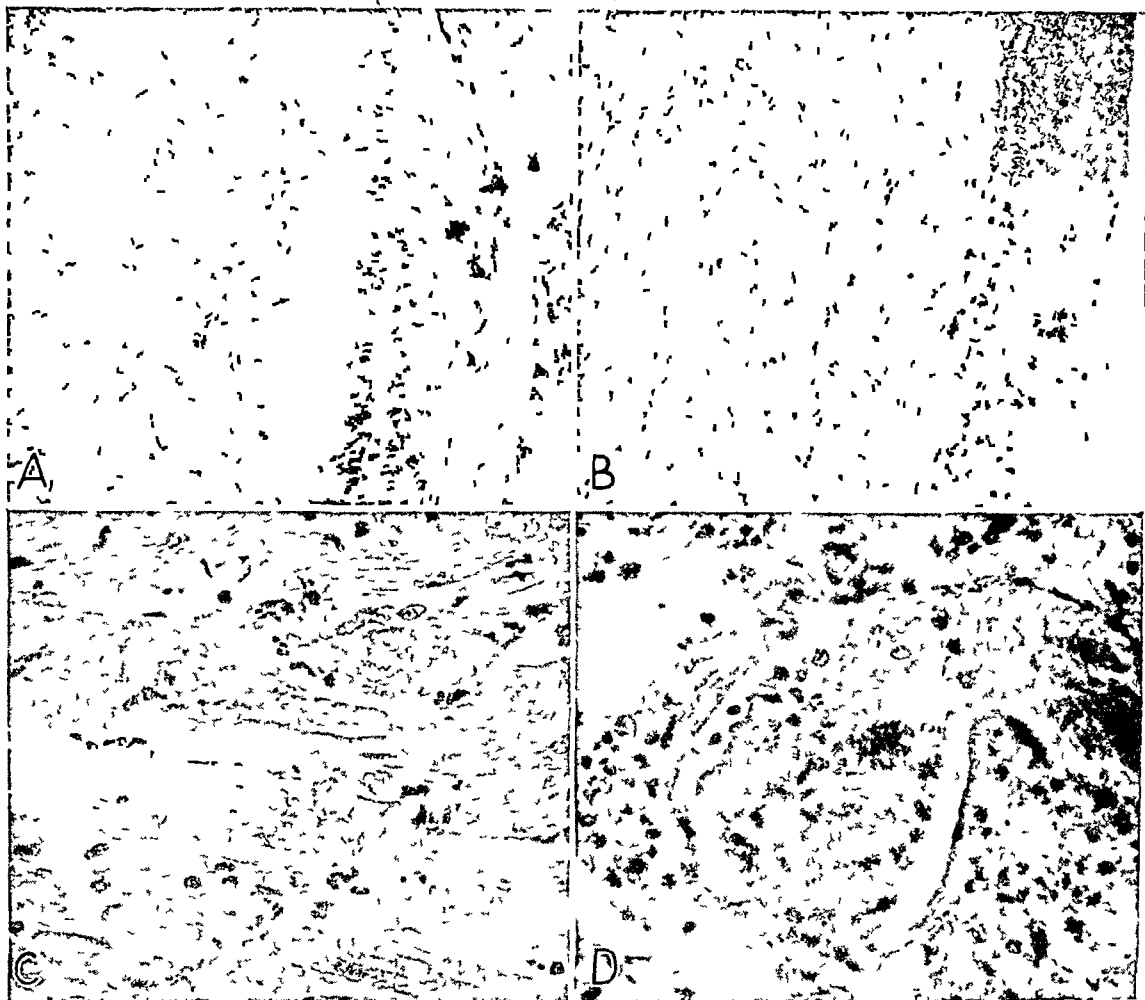


Fig 4—Cardiac and pulmonary lesions occurring in dogs in which hypertensive cardiovascular disease developed following bilateral nephrectomy. *A* (dog 17, survival, $7\frac{1}{2}$ days, blood pressure, 150, 1 dialysis), necrosis of the myocardium near the endocardium. A blood vessel near the center of the necrotic area shows degeneration and necrosis of the media. $\times 42$.

B (dog 14, survival, $8\frac{1}{4}$ days, blood pressure, 160, 2 dialyses), subendocardial hemorrhage and subjacent necrosis of the myocardium. The area of necrosis shows hemorrhage and collection of neutrophils. $\times 59.5$.

C (dog 14, survival, $8\frac{1}{4}$ days, blood pressure, 160, 2 dialyses), higher magnification of necrotic myocardium. Notice transverse hyaline bands and an intervening granular, moth-eaten appearance. The cell boundaries are intact in most fibers, although early coalescence can be seen. At the top, a small blood vessel is plugged with necrotic material. $\times 196.5$.

D (dog 78, survival, 6 days, blood pressure, 160, 1 dialysis), eosinophilic, lumpy, granular embolus in a pulmonary vessel. Nuclear remnants are seen within the embolus. There is no clotting about this structure. Such emboli have suggested an origin from sloughed media of systemic necrotic blood vessels. They have always been seen in smaller branches of the pulmonary vessels. $\times 196.5$.

blood were visible. Usually these pools were surrounded by degenerated fibers. On four occasions the para-adventitial area of smudged vessels of the myocardium displayed prominent numbers of "myocytes."

The foci of myocardial degeneration and necrosis were most commonly encountered near the endocardium and adjacent to the areas of subendocardial hemorrhages. However, they were also observed within the wall of the ventricle several millimeters from the endocardium.

It is evident that the changes described in the foregoing paragraph differ markedly from the accepted picture of myocardial infarction as ordinarily encountered in coronary atherosclerosis. There is no coagulation necrosis, disintegration of fibers constituting the main change. Moreover, the changes observed in isolated fibers support the concept of primary myocardial degeneration and subsequent necrosis.

Smooth muscle tissue other than that of vessel walls also displayed prominent changes. These changes simulated the changes of the vessels occurring before smudging, but with additional variations. The most common site of involvement was the intestinal tract, where the circular and longitudinal layers and the fibers of the villi were affected. In the circular and longitudinal layers the process either was diffuse, involving the entire circumference or sectors, or was in the form of transverse bands involving single fibers or groups of fibers.

The individual fiber changes also consisted of swelling of the sarcoplasm with a hyaline eosinophilic transformation and pyknosis. At times, the entire fiber became prominent, while at other times there were focal bulbous swellings. On occasions, the nuclei disappeared focally. Smudging was a very rare finding, but on occasions polymorphonuclear leukocytes infiltrated the necrotic areas. In some instances there seemed to be dissolution of the muscle fibers and replacement by pools of blood. In the area of intussusception these changes plus classic hemorrhagic infarction were noted.

The intestinal changes described comprise the most commonly observed lesions. As a rule, they were more pronounced in the large bowel, where in some instances the bands of hyalinized fibers resembled foci of severe contraction. In other instances, the foci of hyalinization appeared as oval swellings between which the normal-appearing muscle was narrow, giving the entire layer a sausage-like configuration. Smooth muscle changes of a similar but less intense type were observed less commonly in the stomach, the urinary bladder, the lower part of the esophagus and the aorta.

Degeneration of skeletal muscle was encountered sufficiently often to warrant mention. However, the frequency did not approximate that of the lesions in the intestines and the heart. They were observed in random sections of skeletal muscle, esophagus and diaphragm. On occasions scattered, isolated fibers at these sites showed a loss of cross striation and a lumpy granular or hyaline change of the sarcoplasm. Occasionally the fiber changes resembled Zenker's degeneration.

The aforementioned morphologic changes, observed in part or completely in different animals, appear to constitute the morphologic pattern associated with absence of renal tissue. It is of extreme interest that the same pattern was observed in the group of animals not aided with the artificial kidney as well as in those whose survival was extended by *in vivo* dialysis.

In table 2 the frequencies of the specific lesions associated with this pattern are listed. It is to be noted that most of the types of lesions listed occur in both groups, but with a variation in frequency in the two groups. Moreover, the intensity of the lesions, as a general rule, became more pronounced as survival of the animals was extended.

The bronchial and bronchiolar smooth muscle and the pulmonary arteries and arterioles were closely studied in each animal. These structures were normal in appearance in all instances, thus conforming with established opinion and at the same time serving as an additional control of the observed changes in other tissues.

The smooth muscle of the splenic trabeculae displayed the same changes as smooth muscle elsewhere in animals surviving for longer periods. The central arteries of the splenic corpuscles, on the other hand, revealed no consistent changes.

Controls—As controls, the tissues of 25 normal dogs were studied. All the tissues were normal in appearance, and in no instance were any of the lesions

TABLE 2—*The Incidence of the Various Lesions Observed by Random Sampling in Bilaterally Nephrectomized Dogs**

Period of survival, days	Group 1 Not Subjected to Artificial Kidney		Group 2 Subjected to Artificial Kidney Once				Group 3 Subjected to Artificial Kidney Twice	
	3 to 4+	5 to 5+	4 to 4+	5 to 5+	6 to 6+	7 to 7+	8 to 8+	9 to 9+
Dogs	14	13	4	6	8	13	4	7
Heart								
Subendocardial hemorrhage	21	46	25	16	25	70	25	57
Myocardial hemorrhage	35	38	25	30	50	38	37	43
Isolated degeneration	7	7	0	0	0	15	25	14
Necrosis	0	23	0	0	0	23	37	43
Arteriolar smudge	7	23	0	16	37	53	25	28
Stomach								
Hemorrhage	7	7	0	30	25	30	25	43
Necrosis	7	16	0	15	25	30	0	14
Arteriolar smudge	0	0	0	0	12	7	0	28
Smooth muscle degeneration	7	7	0	15	25	30	0	14
Intestine								
Hemorrhage	0	7	0	0	0	0	0	28
Necrosis	7	7	0	16	37	7	0	14
Arteriolar smudge	7	0	25	16	25	7	25	28
Smooth muscle degeneration	78	84	50	50	75	61	100	57
Esophageal and skeletal muscle degeneration	0	0	0	0	0	7	0	14
Urinary bladder								
Hemorrhage	0	7	0	0	0	15	25	0
Smooth muscle degeneration	7	14	0	0	12	7	25	0
Brain, hemorrhage	21	46	0	0	37	15	50	14
Spleen, smooth muscle degeneration	7	7	0	0	12	38	50	0

* Each number represents the percentage of all animals affected by the lesion listed in the column at the left.

described in the foregoing pages simulated. The control group included animals with intact kidneys subjected to the artificial kidney, animals receiving large doses of heparin, animals whose tissues were allowed to autolyze, and tissues from dogs succumbing to oligemic shock. In no instance were the arterial and cardiac lesions observed. However, in the case of an occasional animal that survived for 24 to 48 hours in shock after freezing of one hindlimb, the smooth muscle changes were demonstrated in the bowel, usually hyaline swelling and pyknosis in the form of the transverse bandlike arrangement. In connection with this finding, it is of interest that the animals had damaged kidneys and a large bulk of necrotic tissue in contact with the circulation. The intestinal smooth muscle changes, as already indicated, are the most common finding in bilaterally nephrectomized dogs and

have been observed as early as three days postoperatively. Moreover, intussusception has also been observed in dogs subjected to procedures leading to oligemia.

The ablated kidneys of each animal showed normal blood vessels and parenchyma. As already mentioned, an additional control was afforded by the lungs of each animal.

COMPARISON OF LESIONS OBSERVED IN BILATERALLY NEPHRECTOMIZED DOGS AND IN PERSONS DYING OF HYPERTENSIVE CARDIOVASCULAR DISEASE

The generalized muscle lesions occurring in the dogs that had undergone bilateral nephrectomy have also been observed in human cases of hypertensive cardiovascular disease and severe bilateral renal disease. Six such cases are being summarized here to emphasize the fact that the observations in the dog have a clinical counterpart.

In man, the medial necrosis of arteries and arterioles characteristic of hypertensive vascular disease has long been known. However, the less advanced hyaline swelling of sarcoplasm and pyknosis of nuclei of medial smooth muscle fibers have not been stressed. Conceivably the "hypertrophy of the media" described in the literature constitutes, at least in part, these degenerative alterations. Our observations indicate that the smooth muscle changes of the gastrointestinal tract, esophagus, urinary bladder, prostate gland and gravid uterus occurring in the course of the hypertensive state or severe bilateral renal disease in man are identical with the changes produced in the dog. The acute degeneration of myocardial and skeletal muscle has been encountered only a few times in human material, but when present it is identical with that observed in the experimental animal. However, it is not unusual to observe focal myocardial scars in hypertensive subjects without a satisfactory degree of coronary disease to account for such scarring. In addition, the prominence of heart failure during the course of clinical hypertensive cardiovascular disease cannot be adequately explained on the basis of simple cardiac strain secondary to elevation of arterial pressure.

CASE 1—A white housewife aged 28 years was known to have been hypertensive four years previously, at which time she was pregnant. The pregnancy terminated in a stillborn infant at term. At the time of admission her complaints were blurring of vision, frontal headaches, nausea and vomiting, easy fatigability, swelling of the feet and ankles and dyspnea on exertion. Four days before admission these symptoms became exaggerated and she vomited a small amount of blood. One brother had died of hypertensive cardiovascular disease at the age of 28 years, and the father was known to have the same disease.

Physical examination revealed nothing except narrowing of the retinal arteries, without hemorrhages or exudates in the fundi and cardiac enlargement. The blood pressure was 250 systolic and 150 diastolic. The specific gravity of the urine was 1.020. Blood urea was 32 mg and creatinine 1.6 mg per hundred

cubic centimeters, and the phenolsulfonphthalein excretion was 60 per cent in two hours. Following a right sympathectomy (Smithwick procedure), the blood pressure was 210 systolic and 150 diastolic. The patient recovered from the operation satisfactorily and was discharged two weeks after the operation to return at a future date for sympathectomy of the opposite side. On the day following discharge the patient was readmitted because of pain of the left side of the chest, numbness of the left arm, abdominal pain and dyspnea. The blood pressure was 244 systolic and 140 diastolic, the pulse rate 110. The following day the patient died after a period of extreme dyspnea, tachycardia and hypotension.

At autopsy the pericardial sac was markedly distended with blood (cardiac tamponade). The hemorrhage was secondary to rupture of a dissecting aneurysm of the arch of the aorta. There were no intimal tears of the aorta. The heart was enlarged, weighing 500 Gm. The left ventricle was 2 cm in thickness. Near the apex there were multiple linear subendocardial hemorrhages.

Microscopically, the media of the aorta showed advanced degeneration of the smooth muscle (hyaline swelling and pyknosis). Near the medial hemorrhage there were clear granular areas in the media (Erdheim's degeneration), with fibrosis of the media elsewhere. The heart showed definite hypertrophy.

In the abdominal viscera hyperemia was prominent, and there was moderate arteriolar sclerosis. The smooth muscle of the gastrointestinal tract showed hyaline swelling of the sarcoplasm and pyknosis. A random section of skeletal muscle revealed degeneration and necrosis of the muscle fibers. The kidneys displayed recent pelvic hemorrhages. Focal scars including hyalinized glomeruli and atrophic tubules were dispersed irregularly throughout the parenchyma. The main bulk of the renal parenchyma was intact. The proximal tubular segments revealed a prominent number of multinucleated epithelial cells.

CASE 2—A white man aged 60 years, of enormous proportions (weight over 300 pounds [136 Kg]), had experienced orthopnea at night for three years and increasing shortness of breath on exertion. He had partaken excessively of food and alcoholic liquors for many years. On admission the patient was lethargic and had bubbling rales at the lung bases, an enlarged heart and massive edema of the lower extremities. The blood pressure was 200 systolic and 110 diastolic. The fundi demonstrated contracted arteries. The blood urea amounted to 32 mg per hundred cubic centimeters. The patient responded poorly to therapy and died two days later.

At autopsy the heart was extremely enlarged, weighing 710 Gm. The average thickness of the left ventricle was 2.2 cm, that of the right, 0.7 cm. The left ventricle had a prominently cupped cavity. The major portion of its subendocardial zone, especially in the apical region and the papillary muscles, showed grayish streaking of the classic tabby cat appearance. The coronary arteries demonstrated minimal atherosclerosis but were grossly patent throughout.

The gross tabby cat appearance was found microscopically to be due to areas of subendocardial degeneration and necrosis of myocardial fibers. The fibers demonstrated lumpy granular changes, focal transverse hyaline accumulations in the sarcoplasm, pyknosis and absence of nuclei of the type observed in the hypertensive dogs. The subendocardial connective tissue was thick and fibrous. Additional findings included marked myocardial hypertrophy, hyperemia, interstitial edema, focal capillary hemorrhages and degeneration of the smooth muscle of arteries and arterioles. Irregular microscopic scars were scattered throughout the myocardium. The apexes of the papillary muscles contained slight deposits of calcium salts. The aorta demonstrated fibrosis of the media and a decrease in smooth muscle fibers.

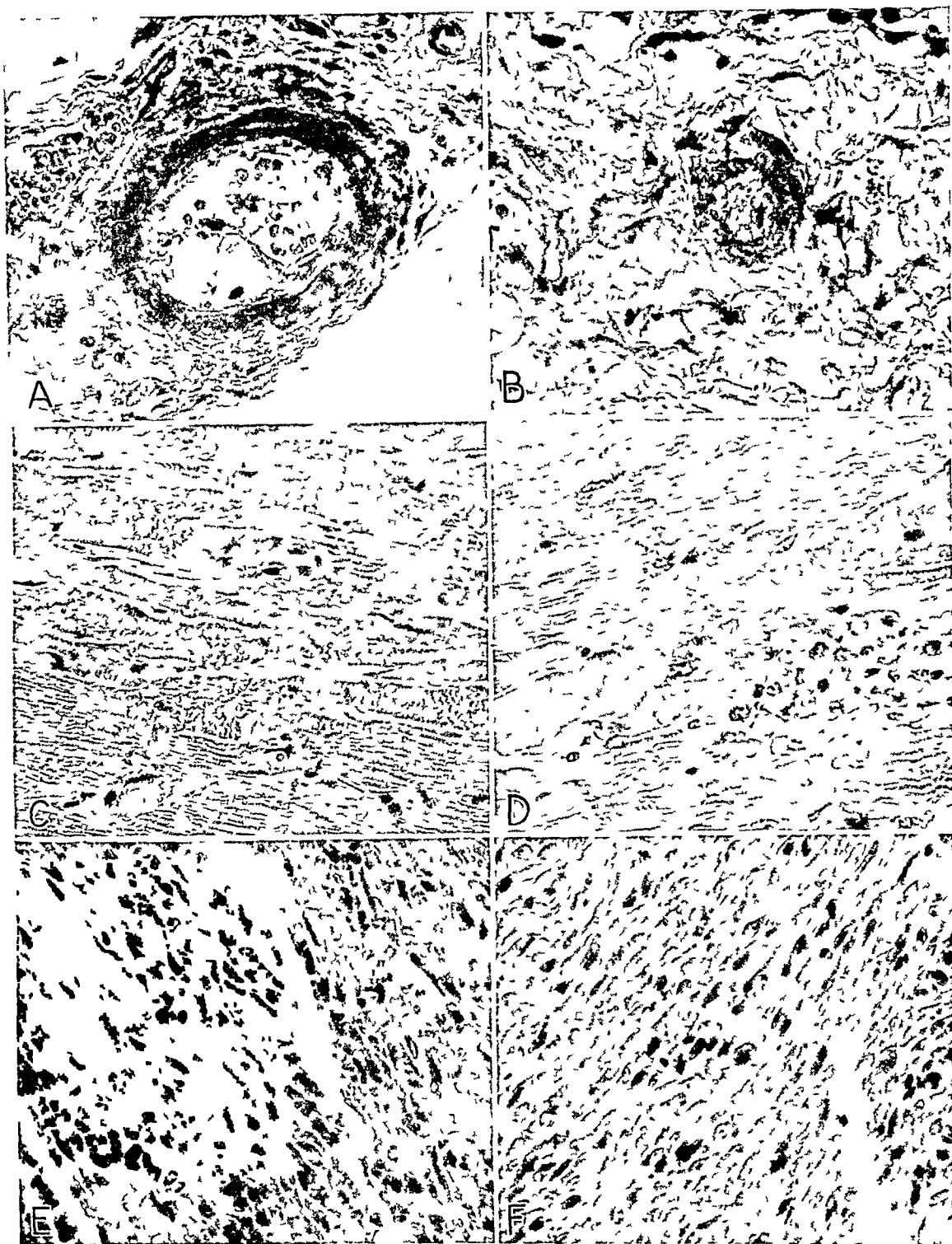


Fig 5—Lesions of blood vessels, heart and muscle as observed in human cases of hypertensive cardiovascular disease *A* (case 5), arteriole in the submucosa of the ileum showing necrosis and smudging of the media identical with that observed in the dog as depicted in figure 2 *E* There are also two lipid-containing macrophages in the intima which are elevating the endothelium $\times 196.5$

B (case 6), arteriole near the capsule of the liver showing medial necrosis and smudging $\times 196.5$

C (case 2), myocardium near the endocardium displaying degeneration and necrosis similar to that encountered in the dog (fig 4 *C*) $\times 196.5$

D (case 1), isolated degeneration and necrosis of skeletal muscle fiber $\times 196.5$

E (case 5), hyaline swelling and pyknosis of smooth muscle fibers of the circular muscular layer of the ileum $\times 196.5$

F (case 4), smooth muscle layer of the esophagus showing marked hyaline swelling of sarcoplasm and pyknosis, as well as focal absence of nuclei $\times 196.5$

The right lung weighed 715 Gm, the left 430 Gm. The main finding included hyperemia, edema and focal hemorrhages. The liver weighed 2,250 Gm and showed advanced portal cirrhosis with fatty metamorphosis.

The combined weight of the kidneys was 570 Gm. The capsule stripped readily, and the surface was fairly smooth. Microscopically, there were small focal scars containing hyalinized glomeruli and atrophic tubules. The main bulk of the parenchyma was intact. The proximal segments contained a prominent number of multinucleated epithelial cells. Few hyalinized arterioles were seen, but an occasional arteriole showed medial necrosis. In the pelvis, hemorrhages were prominent, with several necrotic and smudged arteries and arterioles.

The smooth muscle of the gastrointestinal tract and the urinary bladder showed hyaline swelling and pyknosis. In the pancreas and the spleen arteriolar sclerosis consisted of intimal proliferation and medial hyalinization.

CASE 3—A 45 year old white man, a factory worker, first found to have hypertensive cardiovascular disease eight months prior to admission, entered the hospital complaining of throbbing headaches, nosebleeds, blurring of vision, shortness of breath, "asthma," polyuria and nocturia. Six months previously he had suffered a "stroke," which was followed by weakness of the left side. Four days before admission, gross hematuria developed. Physical findings included enlargement of the heart, determined by percussion, and a loud apical systolic murmur. The blood pressure was 242 systolic and 150 diastolic. Ophthalmoscopic examination revealed hemorrhages, exudates, papilledema, arteriovenous nicking and narrowing of the arteries. The blood urea amounted to 71 mg and creatinine to 3.4 mg per hundred cubic centimeters. An excretory urogram was normal. After unilateral sympathectomy (Smithwick procedure), the blood pressure dropped to 90 systolic and 70 diastolic, the patient became unresponsive, and death followed on the second postoperative day.

At autopsy the heart weighed 720 Gm, with the left ventricle enlarged to 2 cm in thickness. A roentgen study of the coronary arteries by Schlesinger's technic revealed no major obstruction, and grossly the coronary arteries were free of atherosclerosis. Microscopically, the hypertrophied myocardium displayed scattered irregular scars, hyperemia and interstitial edema. Branches of the coronary artery revealed hyaline swelling of sarcoplasm and condensation of nuclei of the smooth muscle of the media.

The lungs weighed 700 Gm and showed hyperemia, edema, focal hemorrhages and pigmented macrophages. The liver weighed 2,300 Gm and was hyperemic, as were also the kidneys, which together weighed 360 Gm. Their surface was moderately granular. Microscopically, there were multiple scars and areas in which atrophy of the parenchyma alternated with hypertrophy. The larger arteries revealed the intimal thickening of arteriosclerosis and hyaline swelling and pyknosis of the medial smooth muscle. Hyalinized arterioles were common. Focal necrosis of the media of arteries and arterioles with eosinophilic smudging was also encountered.

The smooth muscle fibers of the gastrointestinal tract, esophagus, aorta, urinary bladder and prostate gland had undergone a striking degree of hyaline thickening of the sarcoplasm and nuclear condensation and distortion. A peri-adrenal artery revealed necrotic smudging of the media.

CASE 4—A 67 year old white man was unconscious on admission, at which time his blood pressure was 260 systolic and 150 diastolic and the spinal fluid grossly bloody. The patient had suffered two "strokes," one and five years before admission. The patient died the day following admission.

At autopsy the heart weighed 375 Gm, with the left ventricle 1.5 cm in thickness. The coronary arteries were patent. Multiple irregular scars were seen in the microscopically hypertrophied myocardium.

The lungs were moderately emphysematous with focal capillary hemorrhages. The smooth muscle of the gastrointestinal tract and the urinary bladder displayed a striking degree of hyaline swelling of sarcoplasm and pyknosis and distortion of nuclei. The kidneys were small and granular, weighing together 200 Gm, and displayed areas of focal scarring and marked arteriolar hyalinization. The smooth muscle fibers of the media of the larger arteries were thickened and hyalinized. There was a hemorrhage in the left frontal lobe, which had ruptured into the subarachnoid space.

CASE 5—A 47 year old Negro was alleged to have been well until the day before admission when severe headache developed while he was at work and he became unconscious. On admission the blood pressure was 220 systolic and 180 diastolic. The spinal fluid was bloody, and there was paralysis of all the extremities except the right hand. The blood pressure subsequently fell to 170 systolic and 140 diastolic, the patient became cachectic despite the fact that he was fed by tube a high caloric diet, and death followed forty-eight days after his admission.

At autopsy the heart weighed 470 Gm, with the left ventricle 2.5 cm in thickness. The coronary arteries were grossly patent. There was fibrous thickening of the endocardium with hypertrophy of the myocardial fibers. In the myocardium arterial branches demonstrated degeneration and focal necrosis of smooth muscle with early smudging.

The arterioles of the abdominal viscera were hyalinized and thickened. The smooth muscle of the gastrointestinal tract, urinary bladder and prostate gland displayed marked hyaline swelling and pyknosis of the fibers. The kidneys, which together weighed 435 Gm, had a smooth surface but microscopically showed areas of scarring. In both the proximal and the distal segments there were many multinucleated epithelial cells. The smooth muscle of the larger arteries was degenerated. In the brain a hemorrhage was present in the right basal ganglions.

CASE 6—A 49 year old white man suddenly experienced nausea, vomiting and left-sided clonic convulsions. One year before, he had been hospitalized for myocardial infarction and cerebral embolism. On admission there was intense tachycardia and the blood pressure was 150 systolic and 100 diastolic. Subsequently the blood pressure was observed to be 118 systolic and 80 diastolic. There was evidence of myocardial damage. The patient appeared to improve gradually but died suddenly nineteen days after admission.

At autopsy the heart, which weighed 450 Gm, contained a large recent infarct of the left ventricle with a mural thrombus. The descending branch of the left coronary artery was occluded by a recent thrombus near its origin. The myocardial fibers were hypertrophied and contained scattered scars.

The lungs showed hyperemia and multiple confluent parenchymal hemorrhages. The liver weighed 1,320 Gm and had many necrotic arteries and arterioles. These vessels showed the eosinophilic smudging of the media observed in the dog. The pancreas contained both hyalinized arterioles and necrotic arteries and arterioles.

The left kidney weighed 270 Gm and was surrounded by a hemorrhagic envelope 0.4 cm in thickness. On section this kidney had multiple infarcts which had destroyed 75 per cent of the renal substance. The right kidney weighed 280 Gm. Its capsule was densely adherent and the surface granular. There

was a small hemorrhage in the upper portion of the pelvis. Microscopically, infarcts were identified in the left kidney. The right kidney had multiple scars containing atrophic parenchyma. Arteriolar sclerosis with hyalinization of the entire wall was common. In the hemorrhagic area in the pelvis were several small arteries displaying necrosis and eosinophilic smudging of the media.

We believe that this patient primarily had coronary artery disease with myocardial infarction. The infarcts of the left kidney, due to emboli from the mural thrombus, plus nephrosclerosis of the right kidney, apparently so depleted the amount of functioning renal tissue as to be followed by necrotizing lesions of arterioles in the viscera. The findings in this patient are comparable to those observed in the nephrectomized dog.

COMMENT

The hypertension that developed in bilaterally nephrectomized dogs obviously could not have resulted from elaboration of renal pressor substances. It must be concluded, on the other hand, that the kidney normally exerts a function concerned with the maintenance of the normotensive state. In order for bilaterally nephrectomized animals to manifest hypertension of a significant degree, two conditions must be satisfied, namely, the general condition of the animal must be relatively good, and the survival period must be extended to five days or more. In the present study these requirements were attained by depriving the animals of electrolytes and by the periodic application of the artificial kidney.

The present study has demonstrated that survival of nephrectomized animals is limited as a result of the development of widespread lesions, even when the animals are subjected to *in vivo* dialysis. The alterations observed in arteries and arterioles satisfy the established criteria for the changes occurring in hypertensive cardiovascular disease. The observed widespread lesions involved predominantly muscular tissue. In the smooth muscle tissue of the arteries the changes appeared to undergo a transition from degenerative alteration to necrosis. The degenerative changes were characterized by an eosinophilic hyaline swelling of the sarcoplasm and pyknosis of the nuclei. The necrosis was characterized by eosinophilic smudging. The smudged media may spread toward the lumen or toward the adventitia. With the former, narrowing or sloughing into the lumen occurs, the slough at times giving rise to plugging of the capillaries. The spread toward the adventitia imparts to the vessel an accentuated thickness. This vascular necrosis was distinctly segmental in distribution whereas the degenerative changes assumed a more widespread distribution. Normal-appearing vascular segments could be identified in all cases.

Degeneration and necrosis of heart muscle reflected severe injury of this tissue. The fact that this change occurred in isolated fibers indicates that the muscle fiber was altered primarily and was not affected

merely as a result of the vascular changes. The severe damage of the heart might be considered to have interfered subsequently with the attainment of maximal rises of blood pressure.

Other muscular structures involved by the process included the gastrointestinal tract, which was particularly affected. Here the lesions resembled those of the blood vessels in their early phases and those of the heart. A high incidence of intussusception was associated with these muscle changes, and it seems highly probable that there is a causal relationship between these findings. Intussusception was also observed by Winternitz and associates^{4b} and by Goldblatt¹ in dogs with high blood pressure.

Other organs containing smooth muscle were also involved. The urinary bladder, which presumably was in a nonfunctioning state, demonstrated these changes, but to a lesser degree. The involvement of skeletal muscle, although definite, was less widespread.

Study of human tissues, as illustrated in the reported cases, has demonstrated the occurrence of lesions identical with those encountered in the dog with hypertensive vascular disease following bilateral nephrectomy. The degeneration and necrosis of the smooth muscle of arteries and arterioles has been a common finding. Almost equal in frequency have been the similar changes observed in the gastrointestinal tract. Only isolated examples of the acute degeneration and necrosis of myocardium and skeletal muscle have been seen in 100 cases of hypertensive vascular disease which we have examined. However, myocardial fibrosis in the absence of advanced disease of the coronary arteries is common and reflects, we believe, the existence of primary myocardial damage, which accounts for the high incidence of congestive heart failure in cases of hypertensive cardiovascular disease.

The degeneration of smooth muscle has been encountered in both the benign and the malignant forms of hypertensive cardiovascular disease. In the malignant form the necrosis with eosinophilic smudging occurs. As in the experimental animals, the latter change appears to be a more advanced form of the hyaline swelling and pyknosis. Lesions of smooth muscle have also been observed in bilateral renal disease other than hypertensive cardiovascular disease. Outstanding in this group has been glomerulonephritis, either the acute fulminant proliferative type or the chronic destructive type. Also, occasional patients, not exhibiting hypertensive cardiovascular disease clinically but having generalized arteriolar sclerosis and an enlarged heart, have been shown to have degeneration of the smooth muscle of the gastrointestinal tract. Thus, the smooth muscle changes seem to be a counterpart of the arteriolar disease which also results from involvement of this tissue in the blood vessels.

Under certain conditions the smooth muscle changes, particularly those involving the gastrointestinal tract, have been observed in the absence of a terminal elevation of blood pressure, namely, after prolonged peripheral circulatory failure and in the aged with generalized arteriolar sclerosis. In the former condition there is severe renal failure which simulates nephrectomy. In the latter there may be hypertrophy of the heart at the time of autopsy, and these persons may be considered to have had a period of elevated blood pressure, which has receded. In the absence of cardiac hypertrophy these changes in the smooth muscle occur particularly in the aged with generalized arterial and arteriolar sclerosis.

A common finding in subjects of high blood pressure, both young and old, has been the presence of multinucleated cells in the proximal segment of the renal tubule. This alteration has been observed in patients having an adequate renal excretory function shortly before death. It is conceivable that this nuclear proliferation¹⁸ is a reflection of functional alteration of the tubular cells.

In 4 of the 6 human cases reported in this study, severe vascular hypertension was associated with generalized lesions of muscle but not with extensive renal arteriolar sclerosis, in keeping with the observations of Castleman and Smithwick.¹⁹

SUMMARY AND CONCLUSIONS

In dogs bilateral nephrectomy resulted in the development of hypertensive cardiovascular disease when the animals were maintained in a relatively good state of health for a sufficiently long period. At autopsy these animals displayed the vascular lesions commonly ascribed to "malignant hypertension." In addition, the disease process affected muscle tissue throughout the body. The alterations in muscle tissue were of a degenerative and necrotic type and were identical with those seen in the human being dying of hypertensive cardiovascular disease. A detailed study of these lesions observed in the experimental animal and in the human patient is presented.

18 We have designated this change as "tubular nucleosis" in the analogy to the "nucleosis of skeletal muscle." Harman and Hogan (*Arch Path* **47** 29, 1949) have described it as an accumulation of multinucleated epithelial cells occurring in conditions in which hypertension is notable.

19 Castleman, B., and Smithwick, R. H. *New England J Med* **239** 729, 1948.

PERITONEAL PSEUDOMYXOMA

A Report of Four Unusual Cases

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SINCE Werthe¹ first described peritoneal pseudomyxoma in 1884, approximately 500 cases have been reported, in better than four fifths of these the condition was thought to be of ovarian origin. It is the purpose now to describe 3 cases in which it was of appendical origin, with unusual findings in the patients, who were all males. A fourth case, in which the patient was a woman with an unusual history, is included.

The disease is relatively rare. Weaver² reported that in a series of 256 cases of mucocoele of the appendix, the mucocoele was associated with peritoneal pseudomyxoma in 0.11 per cent. He also reported a series of 6,223 appendectomies with only 7 mucocoeles, of which 1 alone had given rise to peritoneal pseudomyxoma. In over 8,100 consecutive autopsies at Montefiore Hospital since 1917, there were only 3 that disclosed this disease. To these 3 may be added a fourth, diagnosed at laparotomy, there was no autopsy in the case. In this series of autopsies, only 3 unruptured mucocoeles of the appendix were noted.

The commonly accepted genesis of the disease, described first by Olshausen,³ is that a cystic dilatation of the appendix (mucocoele) occurs from occlusion, probably the result of inflammation. The mucocoele then ruptures on the peritoneum, where some of the lining cells are implanted, which in turn give rise to cysts, and these, with the original mucocoele, are thought to be the source of a massive "seeding" of the peritoneum with myriads of cysts. Along with this process, there is apparently a reactive fibrosis which results in thickening of the peritoneum and of the cyst walls.

The question how the cysts arise is not settled. The cellular implantation theory is favored. Koerner⁴ suggests that peritoneal pseudomyxoma, secondary to ovarian cystadenoma, is secondary to implantation of appendical cells from a ruptured mucocoele or an inflamed

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1 Werthe, cited by Krivsky¹³

2 Weaver, C. H. *Am J Surg* **36** 523, 1937

3 Olshausen, R. *Ztschr f Geburtsch u Gynak* **11** 238, 1884

4 Koerner, T. *Deutsche Ztschr f Chir* **237** 158, 1932

and ruptured appendix on a freshly ruptured ovarian follicle bed (corpora hemorrhagica) (case 4) Of more importance from a diagnostic point of view is the question of cancer, since in the 4 cases reported here, surgical specimens proved difficult to evaluate and were usually reported as mucogenic adenocarcinoma This problem will be discussed further

HYPOGLYCEMIA IN PERITONEAL PSEUDOMYXOMA

CASE 1—B C was a 46 year old white shopkeeper with an unremarkable history On his first admission, June 24, 1943, his chief complaints were post-prandial heartburn, recent loss of 10 pounds (4.5 Kg) and gradual increase in the girth of his abdomen for about three months There was moderate distention of the abdomen, and large irregular masses were palpable across the upper part of the abdomen, with shifting dullness in both flanks A smooth, firm, orange-sized mass was felt 5 cm above the anus in the anterior rectal area, but it was not fixed There was moderate pitting edema of both legs All roentgen aspects of the gastrointestinal tract were within normal limits The laboratory results were not remarkable Paracentesis yielded 3,800 cc of a clear yellow fluid containing bits of mucoid material Concentration and cell block examination of this fluid failed to reveal tumor cells A laparotomy disclosed "generalized carcinomatosis involving the entire omentum, peritoneum, and surface of the liver" A biopsy of the omental mass was reported as revealing colloid carcinoma During the next four years repeated paracenteses were necessary, each yielding an average of 4,000 cc of yellowish fluid containing gelatinous material There was a gradual loss of weight with continued distention of the abdomen, but the patient was otherwise without complaints

For six weeks prior to the second admission, May 1, 1946, the abdominal swelling had increased and there was diffuse abdominal pain The swollen abdomen had prominent venous collaterals over the surface There was moderate pitting edema of the legs Fine moist rales were heard over both lung bases A huge, firm, nodular, irregular mass occupied the upper right abdominal quadrant and extended down to the iliac crest and to the left as far as the midhypochondrium Shifting dullness was present in both flanks The mass in the rectal area had increased to the size of a grapefruit

The hemoglobin level was 7.0 Gm per hundred cubic centimeters of blood, the red blood cell count was 2,600,000, the white cell count, 5,700, with a normal differential count At first test the fasting blood sugar amounted to 36 mg per hundred cubic centimeters, at a repeated test it amounted to 38 mg Blood urea nitrogen was 17.7 mg, blood cholesterol 12 mg and serum protein, 4 Gm per hundred cubic centimeters, with the albumin-globulin ratio 1.0 Cephalin flocculation was 4 plus, thymol turbidity, 15 units

Two weeks later the patient lapsed into coma During the reaction the blood sugar was reported as 50 mg per hundred cubic centimeters The patient was given 50 cc of 50 per cent dextrose solution intravenously and recovered in a few minutes He had complete retrograde amnesia for the event Two similar episodes occurred on the withholding of breakfast, each preceded by sweating, chilliness and generalized coarse tremors Because of the emergency of the reaction, the blood was not tested for sugar, but the patient was given 75 cc of 50 per cent dextrose solution intravenously and within two minutes regained consciousness Altogether

the patient had five such episodes over six weeks. The shock episodes ceased as abruptly as they had begun after it was no longer necessary to withhold food for diagnostic studies.

A biopsy of a point where some of the mucinous material had apparently infiltrated the subcutaneous tissue of the abdomen, along the old operative site, was reported again as revealing mucinous adenocarcinoma with marked secretion of mucin. The patient was discharged as improved Oct. 30, 1946, and remained fairly free of symptoms until two weeks before his third admission, March 5, 1947, when he began to vomit a coffee-ground material and experienced severe crampy abdominal pain. On admission his blood pressure was unobtainable and coarse moist rales were heard over the left side of the chest posteriorly. The patient was semicomatose and slightly cyanotic. The results of an abdominal examination were similar to those on previous admissions. He failed to respond to treatment and died twenty hours after admission—approximately four and one-half years after the onset of the abdominal swelling. The clinical impression at the time of death was intestinal obstruction and pneumonia. No laboratory studies were made on this admission.

Autopsy (nine hours after death)—The body was markedly emaciated, with a greatly distended abdomen. The peritoneum was infiltrated throughout with confluent pinhead to pea-sized cysts and large grapelike cysts containing a thick, opaque, whitish gray gelatinous material, some of which lay free in the 5,000 cc of turbid brownish fluid in the cavity. This fluid contained bits of necrotic tissue, fibrin and cellular debris. Over portions of the intestines was a grayish brown, friable membrane. Cysts were present on all the peritoneal surfaces. The greatest bulk of gelatinous material occupied the mesocolon, the terminal 10 cm of the mesentery and the serosa of the ileum. A large grapefruit-sized mass of confluent cysts occupied the pelvis.

The appendix was thickened, measuring 6 to 7 mm in diameter near the distal end, where the lumen was occluded. Within the lumen, near the cecal opening, there was some free gelatinous material, proximal to which the lumen was again occluded. At about the central portion the lumen was moderately dilated and contained gelatinous material. A portion of the wall was lined by the gelatinous masses and communicated with an eroded section of the cecum adjacent to the appendical orifice. The entire appendix was buried in the mass of cysts. Partial compression of both ureters had resulted in moderate dilatation. Except for patchy bronchopneumonia of the lower lobe of the left lung, all other organs were normal.

Microscopic Examination—All sections of intestine showed numerous irregular mucin-containing spaces adherent to, or within, the serosa. Sections of the dilated proximal portion of the appendix (fig. 1) were characterized by an atrophic mucosa, devoid of lining cells, with but a few small glands in a thin submucosa, in which there was moderate round cell infiltration. In places the muscularis had been ruptured and pushed aside, and small cystic spaces formed most of the wall. No lymphoid follicles remained. The occluded portion of the appendix showed thickened and fibrotic walls, with moderate round cell infiltration of all layers, and the mucosa and submucosa were replaced by fibrous tissue.

The cystic masses contained mucinous material staining with Mayer's mucicarmine. An occasional cyst was lined by a single layer of low cuboidal cells containing basal hyperchromatic or reticulated nuclei and prominent nucleoli and in places showed a gradual progression from flattened to cuboidal and columnar cells (fig. 2). No mitotic figures were seen. Most cysts were unlined, and occa-

sionally the lining cells lay free within the lumens. The cysts were separated by fairly thick fibrous tissue septums containing engorged capillaries and a few lymphatic channels. In some areas the septums were hyaline. A few granules of blood pigment and phagocytes were present. The surgical specimens were essentially similar.

The liver (fig. 3) contained numerous dilated sinuses with necrotic cells. In some cases fibrocytes were growing into the necrotic zones. The sublobular hepatic veins were engorged and the central veins slightly dilated. These findings suggested a severe increase in hepatic vein pressure, with resultant distention of sinusoids, disruption of cells and hemorrhage.

CASE 2—J. S. was a 51 year old white cloth cutter. His past history was not remarkable except for "yellow jaundice" in 1940-1941, with complete recovery.



Fig. 1 (case 1)—Appendical lumen showing (1) atrophic mucosa devoid of lining cells, (2) few small submucosal glands (these were the only remaining glands), (3) thinned out muscularis, (4) cystic space infiltrating muscularis and serosa, $\times 58$. Note small cell infiltration of all layers.

He was first admitted to another hospital, Dec. 12, 1945. The chief complaint was swelling of the abdomen for nine months. Two paracenteses had been necessary in the previous three months. The abdomen was markedly rotund, with a fluid wave over the lower portion. No organs were palpable. The hemoglobin was 11.3 Gm., the red blood cell count, 3,940,000, the white cell count, 10,000, with a normal differential count. The blood sugar was 60 mg. per hundred cubic centimeters. The diagnosis was "unlisted tumor of the abdomen." The patient was readmitted to the same hospital five times for paracenteses. A blood sugar level taken during this period was 35 mg. per hundred cubic centimeters. He received a number of transfusions for hypochromic anemia. At laparotomy,

June 14, 1946, the following notation was made "The abdominal cavity was filled with a large amount of gelatinous material, the intestines were matted together, and the individual organs were not recognizable, as they were covered with



Fig 2 (case 1) —Lining cells of a small cyst occurring in a myxomatous matrix, $\times 545$ Note single cuboidal to columnar epithelium, hyperchromatic nuclei and prominent nucleoli and secretory granules

gelatinous masses No surgical therapy was thought possible" A biopsy of the omentum was reported as revealing "mucoid degeneration of the connective tissue (pseudomyxoma peritonei?)"

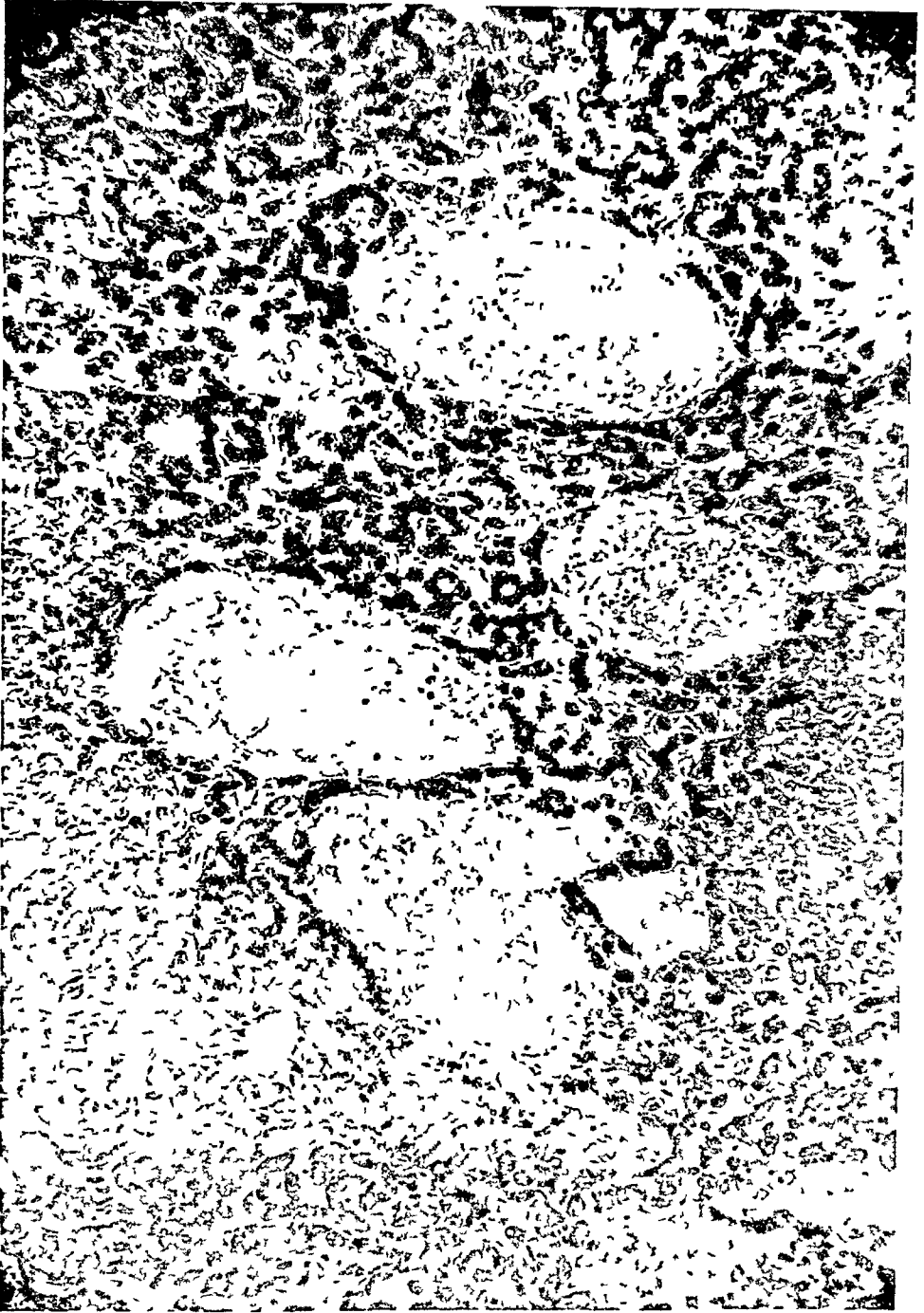


Fig 3 (case 1) —Liver showing multiple areas of disintegration and disruption of cords, blood pools with necrosis of adjacent cells, and dilatation of sinusoids, $\times 83$

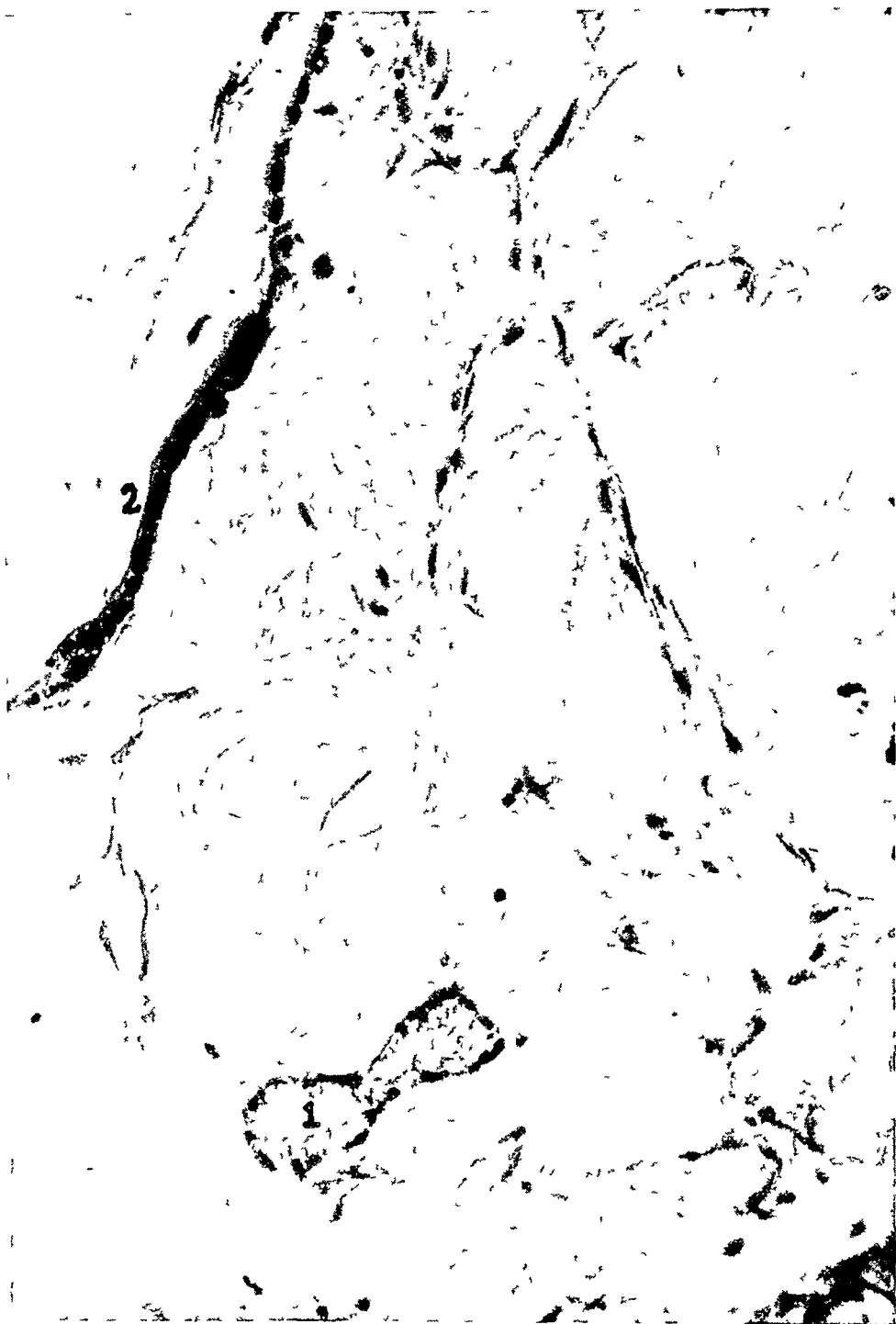


Fig 4 (case 2) —Gelatinous material showing matrix with (1) small active cyst and (2) lining of larger adjacent cyst (see description in text), $\times 255$

The last admission was on Sept 16, 1946, for severe abdominal distention. A fluid wave was present in both flanks and firm irregular masses were palpated across the midabdomen. There was a firm, smooth, orange-sized mass anterior to the rectum. There was moderate pitting edema of both legs.

The hemoglobin was 64 to 95 Gm despite repeated blood transfusions, the total serum protein was 49 to 57 Gm per hundred cubic centimeters, with the albumin-globulin ratio 0.9 to 1.5. The blood sugar varied from 65 to 95 mg and total cholesterol from 134 to 221 mg, per hundred cubic centimeters. The cephalin flocculation test was negative. Retention of sulfobromophthalein was 48 per cent in ten minutes.

Repeated paracenteses and blood transfusions were necessary. On a high calory, high protein diet the edema of the extremities subsided. On Feb 3, 1947, a second laparotomy was done. "Multiloculated cysts filled with gelatinous material occupied the greater peritoneal cavity. The distal end of the appendix ended in such a cystic cavity, and the appendical lumen was filled with gelatinous material. A portion of the cystic mass in the omentum was evacuated, and the appendix and the cyst in which it ended were removed." On the ninth postoperative day pneumonia developed. The patient died nine days later—approximately two and a half years after he had first noticed the abdominal swelling. Unfortunately, no glucose studies were made. Autopsy was not permitted.

Report of Surgical Specimens (Dr S. H. Rosen)—"The specimens were an intra-abdominal cystic mass and the appendix. A cross section of the solid portion of the appendix showed the lumen obliterated by loose fibrillary connective tissue. The propria and the muscularis were thickened by fibrous tissue with marked swelling and separation of the fibers by a homogeneous granular pink-staining material (mucus). There was slight cellular infiltration of all layers. There were large and small intercommunicating irregular spaces filled with a homogeneous granular pink to blue-staining material in the mesoappendix. These spaces were separated by connective tissue septums, which were somewhat vascular and infiltrated in places by inflammatory cells. In some areas the septums were dense and hyalinized. Focal hemorrhage and blood pigment were seen in some areas. The lining (fig 4) of the spaces varied from none to single layered, flattened cuboidal cells, and in other areas the cells varied from single layered cuboidal to columnar cells. These cells had acidophilic cytoplasm, frequently vacuolated, and round central or basal hyperchromatic nuclei, with prominent nucleoli. No mitotic figures were seen.

"A section of the cystically dilated appendix showed a thin wall with swelling, degeneration, and, in places, edema or mucus replacing the muscularis. Slight polymorphonuclear and small round cell infiltration was present. No normal mucosa or lymphoid tissue was seen. The lumen was lined with a single layer of columnar cells similar to those described lining the cysts. A small amount of mucus was present in places on the inner surface. A section of the omental mass showed irregular cystlike spaces exactly similar to those already described." (A review of the biopsy specimen taken at the first laparotomy revealed similar findings.)

SPLENIC INFARCTION AND RUPTURED MUCOCYCLE OF THE APPENDIX

CASE 3—A L. was a 53 year old white laborer. His past history was not remarkable. The first admission was on Feb 15, 1927, with the chief complaint swelling of the abdomen for one month. The abdomen was swollen and rotund,

with a fluid wave in both flanks, a palpable mass of grapefruit size in the left mid-abdominal region and a sliding inguinal hernia of the left side. The hemoglobin was 70 to 80 per cent, the white blood cell count, 2,800 to 3,600, with a normal differential count. An inguinal hernioplasty was made through the open hernial sac. The omentum was a mass of tumors and the peritoneum nodular. Appendectomy was done and a biopsy specimen taken from the omental mass. The pathologist reported metastatic carcinoma and appendicitis.

During the next four years the patient was hospitalized four times with continued swelling of the abdomen and occasional pain in the left upper quadrant. The mass continued to increase in size. There was intermittent diarrhea, and moderate edema of the ankles developed. Repeated paracenteses were necessary, and on two occasions cell blocks of the concentrated abdominal fluid were reported to contain "tumor cells." Gradually dyspnea developed with general weakness, loss of weight, pain in both lower extremities and episodes of nausea and regurgitation. At laparotomy, Feb 11, 1930, the following notation was made: "Large quantities of thick mucoid material were present over the entire peritoneal surface. Intestines and omentum were studded with gelatinous grapelike tumor, and most of the omentum was a thick gelatinous mass. Inoperable."

The final admission was on May 26, 1931. There was no change except that the patient was more emaciated. At the third laparotomy, June 11, this notation was made: "Abdominal findings similar to those found on the second exploration, with the addition of a grapefruit-sized mass in the upper left quadrant. Inoperable." Two days after the laparotomy the patient had severe epigastric pain, rapid pulse and fever. He died four and a half years after the onset of his illness, with intestinal obstruction and possible perforation.

Autopsy (Dr S H Rosen)—The body was that of a fairly well developed, emaciated white man with a large, rotund abdomen, in the left upper quadrant of which a grapefruit-sized mass was palpable. The peritoneal cavity contained 5,000 cc of turbid brownish fluid with numerous yellow and gray friable shreds and dark green flecks. All the peritoneal surfaces were studded with irregular nodules from pinhead to hazelnut size. Some were translucent and composed of firm, thick mucoid substance, others, of firm, opaque, grayish white tissue, and others, of both elements. In places, especially around the descending colon, small nodules were massed in clusters like bunches of grapes. Over the entire parietal peritoneum and over the serosa of the stomach and the large and small intestines they were so closely packed as to form a continuous nodular sheet. Large retroperitoneal cystic masses were present in the region of the descending colon, cecum and appendix (the last having been amputated). Another such mass was found in the rectovesical area. The omentum was greatly thickened and irregular because of the gelatinous and opaque tissue which infiltrated it. The coils of intestine were matted together with a friable grayish and yellowish exudate. In a loop of jejunum was a round perforation about 1 cm in diameter, the edges of which were smooth, soft and reddish green. The plastic exudate appeared more abundant in this area.

The spleen was encased in a thick mass of grayish white opaque, gelatinous material, which completely surrounded it except at the upper pole. This tissue was 3 to 6 cm in thickness and formed an oval mass, measuring 25 by 16 by 13 cm. It was firmly adherent to the parietal peritoneum. On section, about two thirds of the spleen was found to be softened, almost mushy in consistence, and saturated with blood. The markings were obliterated. It was an obvious hemorrhagic infarction. The splenic veins were compressed and occluded near

the hilus by the encasing tumor mass. The entire surface of the liver was covered with large masses of gelatinous tissue which in places extended through the capsule into the parenchyma. Besides the perforation, there was an annular stricture of the ileum about 8 cm from the ileocecal valve. The site of the amputated appendix was marked by an opaque whitish thickening of the wall of the cecum with adherent masses of gelatinous material. All remaining organs were normal.

Microscopic Examination—Numerous sections of the tumor masses showed a greater or lesser accumulation of connective tissue septums, surrounding cystic spaces lined by a single layer of cuboidal to columnar cells. These cells were of medium size, with moderately dense acidophilic cytoplasm and large vesicular or hyperchromatic nuclei and prominent nucleoli. Infiltrating into some of the muscle layers from the serosa of the intestines along the connective tissue septums were a few of these cells forming groups and cords and occasionally pseudoalveoli. In an occasional area there was piling up of the nuclei with loss of cell borders. No mitotic figures were seen. Isolated cells with a tendency toward vacuolation were present. The stroma was loose and fibrillar, with a pale bluish myxomatous appearance.

Sections from portions of the spleen showed sinuses distended with red blood cells, and moderate fibrosis in scattered areas. There was focal necrosis of many of the malpighian bodies. Trabeculi were thickened. In other areas the splenic structure was destroyed and replaced by an almost solid sheet of large, pale mononuclear cells (reticulum and endothelial cells), many of which contained blood pigment. Scattered among these cells were numerous red blood cells and a few lymphocytes.

All other organs were normal.

CASE 4—E. T., a housewife, came to the hospital first in 1930 at the age of 46 because of vaginal bleeding. Hysterectomy and oophorectomy on the left were followed by cessation of bleeding and mild hirsutism. The patient remained well until January 1940, when she began to complain of enlargement of the abdomen. A paracentesis in February 1940 yielded a large amount of gelatinous material. When hospitalized in April 1940 she had a distended abdomen with a fluid wave and a large firm mass in the pelvis. There was a moderate anemia. At laparotomy, April 17, a large amount of thick jelly-like material escaped. A large cystic mass filled the entire pelvis. There were innumerable small cysts in the omentum and peritoneum, and sections of cyst wall showed "papillary pseudo-mucinous cystadenocarcinoma of the ovary."

During the next six years the patient had twenty-two admissions for paracentesis but remained otherwise in fairly good health, complaining only of slight but continuous enlargement of the abdomen and the need for repeated paracenteses. When admitted for the twenty-second time, March 12, 1946, the abdomen was so enlarged that roentgenograms of the chest and an intravenous pyelogram were difficult to interpret.

At laparotomy, April 19, the surgeon removed an immense cystic mass by sharp and blunt dissection, separating it from the bowels and the lateral pelvic walls. Two elongated cystic nodules attached by pedicles to the mesentery were also removed. The patient made an uneventful recovery, and following moderate high voltage radiotherapy there was no recurrence of the mass or the ascites.

July 21, 1947, the patient was suddenly seized with severe colicky epigastric pain which began in the right upper quadrant and was transmitted across to the left upper quadrant of the abdomen. When she was admitted to the hospital

two days after the onset of pain, tenderness was present in all quadrants of the abdomen, with no spasm, distention or increased peristalsis. No masses were noted. Her temperature ranged from 101 to 103 F. There was occasional vomiting. A flat roentgenogram of the abdomen showed some fluid levels in the small bowel. No definite diagnosis was possible. Five days after admission her condition changed rather quickly as her pulse became fast and thready and tenderness developed in the right lower quadrant and costovertebral angle. A diagnosis of peritonitis was made, and she was treated with penicillin and streptomycin, but died six days after admission—one year and three months after the removal of the abdominal mass and seven years after the beginning of her illness. The patient had moderate hypertensive cardiovascular-renal disease and was a chronic complainer of fibrillation, but at no time was she known to have had cardiac decompensation and there was no sign of cardiac failure.

Autopsy (Dr E. M. Blondheim)—The body was that of a well developed, well nourished woman of about 63 years of age. Except for two abdominal scars, there were no abnormalities externally. The parietal peritoneum was markedly thickened and brownish. About 2,000 cc of a thin purulent yellowish fluid was present, and the peritoneum was covered with a greenish fibropurulent exudate. On the under surface of the diaphragm and between the capsule of the spleen and the surrounding tissue there were moderate numbers of pinhead-sized red and blue jelly-like implants. The serosal surfaces of the intestines were covered with a fibrinopurulent exudate. Extending up the medial border of the cecum was the appendix. It was attached to the cecum laterally by fibrinous adhesions and covered with a thick fibrinous-purulent exudate and some fecal material. The latter was oozing out of a punched-out pinhead-sized perforation near the distal end of the anterolateral aspect. The wall was somewhat thickened and fibrotic throughout, and the lumen was not occluded. The pelvic organs were absent, and the pouch of Douglas was obliterated by fibrous tissue. The left side was somewhat deeper than the right and contained a shaggy purulent greenish exudate. The remaining abdominal organs showed no abnormalities.

Microscopic Examination—Sections through the capsule of the spleen at the site of the jelly-like implants revealed islands of a myxomatous material similar to Wharton's jelly. Similar myxomatous tissue was found on the under surface of the diaphragm. A thick layer of edematous fibrous tissue with round cell infiltrate and small deposits of calcium lay beneath the muscularis of the appendix. The lumen was dilated and the mucosal epithelium absent. In one area the mucosa was represented by a few poorly preserved papillary processes in which were a few pale-staining cells with prominent vacuoles. The bases of some of the crypts were recognized and consisted of columnar cells with basal nuclei and occasional goblet cells. The serosal surface showed edematous and inflamed fibrofatty tissue with a surface layer of fibrin and in this region were a few islands of degenerated myxomatous tissue, already described. The other organs were not remarkable.

Review of Surgical Material (Pelvic Cystic Mass—April 19, 1946)—A large irregular mass, measuring 27 by 14 by 7 cm, had coarsely nodular surfaces, owing to multiloculated cysts, which were filled with a glarry greenish gelatinous fluid. The cysts varied from a few millimeters to 7 or 8 cm in diameter, and the walls varied from paper thin to 5 or 6 mm. The walls consisted of gray fibrous tissue with a smooth lining, and the larger walls contained in some areas cysts with mucoid material. Two smaller ovoid cystic masses were present (omental biopsies).

Microscopically, the tumor consisted of large irregular branching tubules and of small and large cysts. The tubules were lined by one to several layers of tall columnar cells. Some of the cysts showed remnants of low cuboidal or flattened lining cells. The lumens contained bluish or pinkish stringy mucinous material mixed in places with fresh blood. The original diagnosis was pseudo-mucinous cystadenocarcinoma.

COMMENT

Hypoglycemia—Two standard oral glucose tolerance tests (50 mg dextrose) during the period of attacks in case 1 (curves 1 and 2 in the tabulation) showed a slow rise in the blood sugar levels from hypoglycemic to low maximum levels at one and one-half and two hours, respectively, followed by a slow fall to the levels seen in four hours in the second test.

	Curve 1 Mg per 100 Cc	Curve 2 Mg per 100 Cc
Fasting level	38	75
½ hr	68	95
1 hr	72	138
1½ hr	80	125
2 hr	120	115
3 hr		90
4 hr		65

Another standard oral tolerance test four months after the attacks had ceased showed a fasting sugar level of 78 mg per hundred cubic centimeters, which rose to 190 mg in one-half hour and fell to 65 mg in three hours—an essentially normal curve. Two epinephrine sensitivity tests during the periods of attack, with 0.5 mg of epinephrine hydrochloride injected subcutaneously, showed fasting sugar levels of 68 mg and 70 mg per hundred cubic centimeters, respectively, with a maximum rise of 10 mg in one-half hour in both tests. Thereafter the levels fell to 65 and 45 mg, respectively, in two hours. Five units of regular insulin, following a fasting sugar level of 70 mg, caused a drop to 60 mg in one-half hour and 40 mg in one hour, after which it was necessary to give the patient dextrose intravenously to prevent shock, as he had begun to show premonitory symptoms. Blood sugar levels at intervals while food was withheld showed a slow decrease from 60 mg per hundred cubic centimeters on arising at 6 a. m. to a low of 40 mg at 1 p. m., when symptoms appeared. The patient went into shock at 2:20 p. m., when the blood sugar level was 48 mg. Unfortunately, no intravenous dextrose tolerance tests were made.

These tests suggest that during the periods of attack, at least, there was some interference in absorption and probably a low glycogen reserve. The possibility that glycogenolysis was interfered with or that insulin sensitivity increased cannot be excluded. Epinephrine caused only a slight rise in blood sugar, and then a slow response. Insulin in a dose of only 5 units caused a slow but definite decrease in the blood sugar and a sustained response.

Among the various mechanisms that may have produced hypoglycemia in this patient, hepatic insufficiency must be considered in view of the changes in the liver, the abnormal results of liver function tests and the role of the liver in the maintenance of the blood sugar level. However, the exact mechanism is obscure. Seckel⁵ reported a case of hypoglycemia in which a large fibroma of the liver was so situated as to exert pressure on the right splanchnic nerves and celiac ganglion, thus presumptively inhibiting sympathetic impulses to the liver. Disruption of sympathetic impulses to the liver has been demonstrated to cause hypoglycemia in cats by Evans, Tsai and Young,⁶ and such a mechanism may have been operating in Seckel's case. Since large masses of tumor were present in the region of the celiac ganglion and right splanchnic chain, one might speculate on the possibility of such a mechanism in case 1. However, there was no evidence of inhibition of glycogenolysis in antemortem and post-mortem studies of the glycogen content of the liver as reported by Seckel. In case 1 the postmortem analysis of the liver for glycogen was reported to have shown 137 mg (of glucose) per hundred grams of tissue. Since the necropsy was made nine hours after death, these figures do not help. Conn⁷ considers interference with the sympathetic innervation of the pancreas and the adrenal glands to be a mechanism of production of idiopathic hypoglycemia in some cases, and such a mechanism might possibly play a role here also, since the mass of gelatinous material surrounding these organs might easily have interfered with innervation.

It is well known that prolonged oxidation, as in strenuous physical exertion, can lead to hypoglycemic episodes. But it has never been shown that a rapidly growing tumor alone can utilize sufficient oxygen and hence glucose to produce hypoglycemia in the presence of an adequate intake and reserves. Woodward and Fry⁸ have shown that the average blood sugar levels in patients with cancer are about 20 mg higher than those in normal controls on the same hospital diet, and that noncancerous growths also tend to be associated with elevated blood sugar levels. One might logically assume that a rapidly growing neoplasm probably induces increased glycogenolysis and hence some elevation of blood sugar, as long as the glycogen stores or glycogen precursors (fat and protein) are not depleted. On depletion of these stores, however, hypoglycemia might ensue. In the presence of

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6 Evans, C. L., Tsai, C., and Young, F. G. *J. Physiol.* **73** 67, 1931

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8 Woodward, G. E., and Fry, E. G. *Biochem. J.* **26** 889, 1932

possible impairment of absorption, hepatic insufficiency and a wasting disease, the demand for glucose by the tumor might well have led to hypoglycemic blood sugar levels

Evensen⁹ and others have shown that often hypoglycemia develops after gastroenterostomy, and he attributes this to rapid emptying of the stomach with subsequent faulty absorption. Although in case 1 the emptying time of the stomach was not abnormal, there is suggestive evidence of faulty absorption in the deficiency pattern of the small bowel on roentgen examination and the abnormal high fat content of the stool (37 per cent of dry weight) on a low fat diet. The low blood cholesterol (90 mg per hundred cubic centimeters) and the low serum proteins (4 Gm per hundred cubic centimeters) could be partially explained on the basis of faulty absorption as well as hepatic insufficiency, certainly loss of protein in the fluid removed by paracenteses played a role. Regardless of the mechanism, the patient was seriously undernourished and, as shown by Gounelle and Marche¹⁰ and others, fatal hypoglycemic shock can develop in undernourished patients with nutritional edemas. Such a mechanism might be all that is needed to explain the present case except that if this were all one might wonder why hypoglycemia is not more often seen in cases of carcinomatosis or other severely debilitating diseases, or in previously studied cases of peritoneal pseudomyxoma.

Other cases in which hypoglycemia was attributed to faulty absorption, as in Frank's,¹¹ that of a boy suffering from ascariasis (which cleared up on adequate antiparasitic therapy), suggest that at least faulty absorption of glucose may play a role in hypoglycemia. The massive involvement of the mesentery in 2 cases reported here may well have inhibited absorption, even though there was no microscopic evidence of obstruction of the mesenteric lymphatic vessels.

It has been suggested further that the pseudomucin, which contains a polysaccharide conjugated with a protein in the mucotin-sulfonic acid portion of the complex, may in some manner bind or convert sufficient glucose to result in hypoglycemia when large quantities of pseudomucin are present. (In case 1 the total gelatinous material weighed approximately 9,000 Gm.) This idea may be worth further study.

In the absence of any conclusive evidence establishing the cause of the hypoglycemia, this is best explained as a combination of faulty absorption, hepatic insufficiency and debilitation resulting in sufficient depletion of glycogen, protein and fat stores that the withholding

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10 Gounelle, H., and Marche, J. *Occup Med* **1** 48, 1946

11 Frank, L. L. *Am J Digest Dis* **11** 195, 1944

of food precipitated episodes of hypoglycemia After six weeks of a high protein diet, and after it was no longer necessary to withhold food for any purpose, the attacks ceased Peripheral edema likewise subsided, although the mass continued to grow In 2 of the other 3 cases reported here there was likewise a disappearance of the peripheral edema on high protein diets

DIAGNOSIS OF PERITONEAL PSEUDOMYXOMA

The diagnosis of peritoneal pseudomyxoma was not made clinically in any of these cases Indeed, in few cases has it ever been so diagnosed Himmelfarb, Ager, Kozinsky, Peters, Mekhedko and others, as reported by Mekhedko,¹² have, however, made the clinical diagnosis Mekhedko stressed clinically the indefinite contour of the abdominal masses, the slowly developing enlargement of the abdomen, the absence of cachexia during the greater part of the course and the small fluctuations and lack of change in the percussional note on change of position of the patient, and, finally, a history of recurrent attacks of abdominal pain (presumptive recurrent appendicitis) In addition, the signs and symptoms stressed by Mekhedko, the silent, nonpainful, nature of the swelling early in the course of the disease, the necessity for repeated paracenteses, the engorged abdominal veins and, finally, the terminal symptoms of intestinal obstruction or perforation (in 3 instances) characterized our cases and closely approximate the clinical course described by Krivsky¹³ and others

However, in most cases the diagnosis, if made prior to death, will be made at laparotomy Yet, despite repeated laparotomies, the nature of the illness was not appreciated in 3 of the 4 cases, being correctly diagnosed at laparotomy only in case 2, and then with assurance only at the second operation Two factors stand out as being responsible for this failure of diagnosis 1 The absence of a demonstrable mucocele, the appendix having been surgically removed prior to the onset of symptoms, as in case 3, or the appendix having been buried in the mucinous mass, as in case 1 (It is of interest that at the time of removal of the appendix in case 3 [four years prior to death] it was reported as inflamed) 2 The reports on biopsy material consistent with colloid or mucogenic adenocarcinoma Case 2 was recognized as one of pseudomyxoma only when a definite mucocele of the appendix was seen

The repeated reports of adenocarcinoma are important, for there is no consistent opinion as to the nature of peritoneal pseudomyxoma, and some have thought that both cancerous and noncancerous lesions

12 Mekhedko, V P Vrach delo 19 889, 1947

13 Krivsky, L A, Jr J Obst & Gynaec Brit Emp 28 204, 1921

can give rise to the gross anatomic picture Krivsky¹³ reported about 7 per cent to be "malignant" histologically, while Woodruff and McDonald¹⁴ reported 38 per cent to be "malignant" Barzilai¹⁵ expressed the opinion that the disease when of ovarian origin is histologically "benign," although clinically "malignant" Woodruff and McDonald¹⁴ distinguished between "nonmalignant" cystic tumors of the appendix and grade 1 adenocarcinoma of the appendix, which they consider the type of growth of the appendix which may rupture and give rise to peritoneal pseudomyxoma. However, their criterion of cancer of the appendix (lining cells forming pseudoalveoli and invading the underlying stroma) cannot be applied in microscopic examination of peritoneal or omental cystic masses in some cases because, for the most part, the cysts are unlined, or if lined with cells, as in cases 1, 2 and 4, these cells may or may not show such pseudoalveolar or invasive characteristics. In cases 3 and 4 such changes were seen fairly frequently, in cases 1 and 2, very infrequently. Rubnitz and Herman¹⁶ have considered the criteria of cancer to be the presence of lining cells alone, those not lined being considered benign. Such criteria help very little, for many of the cysts will be unlined and others lined, and it is a matter of chance whether the particular section examined will have lining cells. Furthermore, within the same section, the lining may vary from flattened to high cuboidal cells with evidence of goblet formation. The decision as to whether or not the condition is shown to be cancer on histologic observation becomes in such cases almost a matter of individual interpretation, since the obvious evidences of cancer are lacking—mitotic figures, metastases, bizarre cell arrangements, etc.

The important point, therefore, is that even in the presence of cells which may be thought by the examiner to be cancerous a diagnosis of peritoneal pseudomyxoma should be entertained when the history is consistent and laparotomy shows a peritoneum infiltrated with gelatinous cysts, even in the absence of a demonstrable mucocoele of the appendix. The finding of a mucocoele should expedite matters at once. A cancerous lesion can very likely produce the gross anatomic picture of peritoneal pseudomyxoma, but microscopically, for the most part, the lesions are relatively benign. Even the benign appearance of the cysts, however, in the presence of consistent gross anatomy is not sufficient evidence to warrant a definite diagnosis of peritoneal pseudomyxoma, for I have studied a case of mucogenic adenocarcinoma (presumptively of colonic origin) which was so diagnosed because it had spread or

14 Woodruff, R., and McDonald, J. R. *Surg., Gynec. & Obst.* **71** 750, 1940

15 Barzilai, G. *Atlas of Ovarian Tumors*, New York, Grune & Stratton, Inc., 1943

16 Rubnitz, A. S., and Herman, K. T. *Arch. Path.* **36** 297, 1943

metastasized to inguinal and mediastinal lymph nodes but which otherwise grossly was similar to the 3 cases of pseudomyxoma reported in the foregoing pages, and histologically indistinguishable. However, some of the early authors (Krivsky¹³, Olshausen³) might well have considered even that a case of peritoneal pseudomyxoma, for in some of the cases they reviewed the patients did have parenchymal involvement and distant metastases. Furthermore, reports describing peritoneal pseudomyxoma originating from the gallbladder, from persistent patent omphalomesenteric duct and other sites suggest that even the demonstration of a mucocele of the appendix or a cyst of the ovary is not necessary to establish the diagnosis. The important point is, therefore, that the diagnosis is essentially a gross anatomic one, and that to rely on the microscopic changes may often erroneously prohibit a diagnosis of peritoneal pseudomyxoma.

A review of case 4 reveals at least three important points. 1 Because of the original diagnosis of papillary cystadenocarcinoma of the ovary with metastases, no real effort was made for six years to reevaluate the case and attempt therapy. Even though it was suggested on the basis of the large amounts of pseudomucinous-like material obtained at paracenteses that this condition might have had a pseudomucinous origin, the term "carcinoma" had been used and the case was considered hopeless. Then, after six years, in which the patient did not deteriorate, it was finally thought advisable to reattempt surgical treatment. The attempt was successful in completely eradicating the disease. 2 Because the possibility of peritoneal pseudomyxoma of appendical origin was not entertained, even at the time of successful surgical therapy, the appendix was not examined, and eventually it became the source of the patient's death. How in this day and age, after two exploratory laparotomies, this patient escaped an appendectomy, I cannot guess, but she did, and for me it has resulted in a dramatic support of Koerner's theory concerning the origin of peritoneal pseudomyxoma in females. This theory holds that the pseudomyxoma is never the result of a primary pseudomucinous cystadenoma of the ovary but is always secondary to a ruptured mucocele of the appendix. Koerner⁴ stated, and is supported by Mekhedko,¹² that a normal appendix has never been reported or demonstrated in a case of peritoneal pseudomyxoma in which the patient was a woman. In at least 25 per cent of reported cases in which the tumor occurred in a woman it had a dual origin imputed to it because in those cases the appendix was examined and a mucocele was found. The lesson to be learned, then, is this. In the presence of a pseudomucinous cystadenoma or cystadenocarcinoma of the ovary with peritoneal implants, one should consider the possibility of peritoneal pseudomyxoma and remove the appendix as well as the tumor, regardless of what the appendix may look like. There is

abundant evidence in the literature, especially the German and Russian, to show that the prolongation of life is greater when both possible sources of the spread of the cystic masses are removed than when the ovary alone is removed. This concept is not stressed in the American literature, and it may well be that a greater awareness of the possibilities will result in greater numbers of surgical cures in cases diagnosed as cystadenocarcinoma of the ovary if the appendix is removed routinely with the ovarian mass, even when there are apparent metastases. Many cases of peritoneal pseudomyxoma must have gone into the records as cases of colloid carcinoma, mucogenic adenocarcinoma, pseudomucinous cystadenoma or adenocarcinoma. Clinically the prolonged course in a case considered hopeless on the basis of pathologic observations and questionable spread or metastases should be the tip-off. Cancers rarely allow a prolongation of life to this extent after spread has occurred, and especially when no treatment, surgical or radiologic, has been attempted. Furthermore, a thorough exploration of the abdomen may reveal that there are no parenchymal metastases, no actual invasions of organs, and this fact should put the surgeon on his guard. The spread of peritoneal pseudomyxoma is entirely along the serous membranes, and if parenchymal involvement occurs, it is rare and probably late in the course. This, then, is a plea for an awareness of the possibility of cure of peritoneal pseudomyxoma as well as a plea to think of it in hopeful terms, and get in early. Do not let a pathologic report scare you out. The disease may be more common than was previously thought, and, indeed, more and more case reports are piling up from year to year.

The importance of arriving at the diagnosis as early as possible must be stressed in light of the fairly numerous reports that life was prolonged by removal of the appendix (and ovary) and as much of the cystic masses as possible (Olshausen,³ Masson and Hamrick,¹⁷ Timoney,¹⁸ Voight,¹⁹ Meleney²⁰ and others). Omentopexy has been thought to be of value when it could be performed. Barzilai¹⁵ and Mekhedko¹² both suggested that postoperative irradiation was of some value, and case 4 seems to confirm this opinion.

CONCLUSIONS AND SUMMARY

Four cases of peritoneal pseudomyxoma are reported. In one, hypoglycemia was an incidental finding, in another hypoglycemia reached shock levels, in the third there was massive infarction of the

17 Masson, J. C., and Hamrick, R. A. *Canad. M. A. J.* **22**: 508, 1930.

18 Timoney, F. X. *Am. J. Surg.* **64**: 417, 1944.

19 Voight, W. W. *Illinois M. J.* **71**: 172, 1937.

20 Meleney, T. L. *Surgery* **103**: 457, 1936.

spleen as a result of compression of the splenic vein and massive encapsulation of the organ with the cystic mucinous growth, the fourth, in which the patient was a woman, was remarkable because of the result obtained with surgical treatment and because death resulted eventually from a perforated mucocele of the appendix

Various possibilities as to the genesis of the hypoglycemia in 2 cases are discussed. It is felt that the best explanation is that a combination of faulty absorption, hepatic insufficiency and debilitation due to mechanical interference with functions of the bowel and the liver probably led to the hypoglycemic attacks

The difficulties of diagnosis and the problem of distinguishing peritoneal pseudomyxoma from mucogenic adenocarcinoma are discussed

It is suggested that more attention be paid to the possibility that in females peritoneal pseudomyxoma may originate in the appendix, even in the presence of an ovarian cystadenoma or an ovarian cystadenocarcinoma, and that the appendix be removed in all cases in which the slightest doubt may exist as to the differential diagnosis. Credence is given to the possibility that even in females the appendix and not the ovary may be the site of origin of peritoneal pseudomyxoma

INFLUENCE OF DICUMAROL® ON STREPTOCOCCIC INFECTION IN RABBITS

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AND

D MURRAY ANGEVINE, M D

MADISON, WIS

IT HAS been demonstrated chiefly by Menkin¹ that fibrin plays an important part in the localization of infection by forming either a fibrinous network in the tissues or thrombi in the lymphatic channels about areas of inflammation. This hypothesis was first tested by studying the rate at which dyes pass into, and out of, inflamed areas. Observations were also made with various bacteria, and it was noted that in localized infections produced by injecting staphylococci and pneumococci into rabbits fibrin was conspicuous in lymphatic channels, whereas it was less evident or absent after injections of hemolytic streptococci. Because of other factors involved in the localization of infections, such as phagocytosis and the "spreading factor," the explanation based on mechanical fibrin blockage has never been accepted without considerable reservation.

To obtain additional information in this connection, it seemed desirable to study the course of experimental infections in normal as well as in dicumarolized animals in which the mechanism of coagulation would be interfered with so as to prevent or decrease the formation of fibrin. By the utilization of such a method it seemed possible that a more adequate answer to this problem might be obtained.

METHOD

Rabbits weighing from 2,000 to 3,000 Gm were selected in pairs, so that the treated and control animals in each experiment were approximately of the same weight. The animals were fasted from twenty-four to thirty-six hours prior to, as well as during, the experiment. Water was offered freely. Dicumarol® (3,3'-methylenebis[4-hydroxycoumarin]) was administered orally in gelatin capsules in doses of 6 mg every other day. Prothrombin time was determined on blood from the ear by the method of Quick. The hair was clipped over one flank, and the rabbit was given an intracutaneous injection of an eighteen hour blood broth culture of hemolytic streptococci (strain H)². The culture was diluted in broth

From the Department of Pathology, University of Wisconsin Medical School

1 Menkin, V. Dynamics of Inflammation, New York, The Macmillan Company, 1940

2 Angevine, D M. J. Exper. Med. 64 131, 1936

so that an inoculum of 0.1 cc contained the equivalent of 0.01 to 0.001 cc of the original culture. The cutaneous lesions of both the control and the treated animals were observed and measured frequently. The measurements represent the average of the two diameters and the height in millimeters. Thus a lesion that measures 40 by 64 by 4 mm is recorded as 52.4. Those in the table represent measurements made at forty-eight hours except when an animal was killed prior to that time. The prothrombin time was usually determined twice on each animal, the greater response only is included in the table. The blood was cultured at the time of death or when the animals were killed. In the first few experiments the animals were infected on the day following that of the first dose of dicumarol®, subsequently they were infected after 3 doses of dicumarol® had been administered over a period of from three to five days. This permitted a more adequate oppor-

Infections in Normal and Dicumarolized Rabbits

Experiment	Administration of "Dicumarol" and Culture, Day						Cc of Culture Injected	Prothrombin Time, Sec	Interval Between Infection and Death	Size of Lesion, Mm		Blood Culture		Fibrin	
	1	2	3	4	5	6				Treated	Control	Treated	Control	Treated	Control
1	D	I	D		D		0.01	67		52.4	26.6	+ d 4	O		
2	D	I	D		D		0.01	60	K 10	60.6	24.7	O	O		
3	D	I	D		D		0.01	120		50.8	26.6	+ d 5	+ d 8		
4	D	I	D		D		0.001	17	K-10	27.6	20.5	O	O	+	+++
5	D	I	D		D		0.001	60		22.3	19.3	+ d 4	O	+	++
6	D	I	D		D		0.001	180		19.4	20.5	+ d 4	O	O	++
7	D		D		DI		0.01	180	K 2	25.3	26.4	O	O	O	+++
8	D		D		DI		0.01	5	K 6	43.4	25.3	O	O		
9	D		D		DI		0.01	180		5.1	32.4	+ d 2	O	O	O
10	D		D		D	I	0.01	33	K 1	35.2	23.4	O	O	+++	+++
11	D		D		D	I	0.01	180	K 2	90.2	24.3	+	O	O	+
12	D		D		D	I	0.01	39	K 3	55.3	60.4	O	O	+	+++
13	D		D		DI		0.01	16	K 1	31.3	21.4	O	O	+	+++
14	D		D		DI		0.01	5	K 3	34.3	28.5	O	O	+++	++
15	D		D		DI		0.01	50	K 3	45.3	37.4	+	O	++	+++

D = "dicumarol"

I = Injection of culture of hemolytic streptococci (strain H)

K 10 = killed after ten days.

d 4 = died after four days

tunity for response to the drug. The variations in these relations are indicated in the table.

Histologic preparations were made of the lesions in most of the experiments. In some instances tissue was removed for study at intervals of twenty-four, forty-eight and seventy-two hours from both treated and control animals. In other instances the lesions were removed for sectioning when the animal was killed. The lymph node draining the site of inoculation was also sectioned. To demonstrate fibrin in the tissues, the sections were stained with Mallory's phosphotungstic acid-hematoxylin.

RESULTS

The most important findings are incorporated in the table. Only 2 of 15 treated rabbits (experiments 8 and 14) did not show an increase

of prothrombin time The response varied widely in different animals (from 16 to 180 seconds) The prothrombin time in normal animals was never more than 5 seconds The cutaneous lesions of dicumarolized animals were appreciably larger than those of controls (figs 1 and 2)

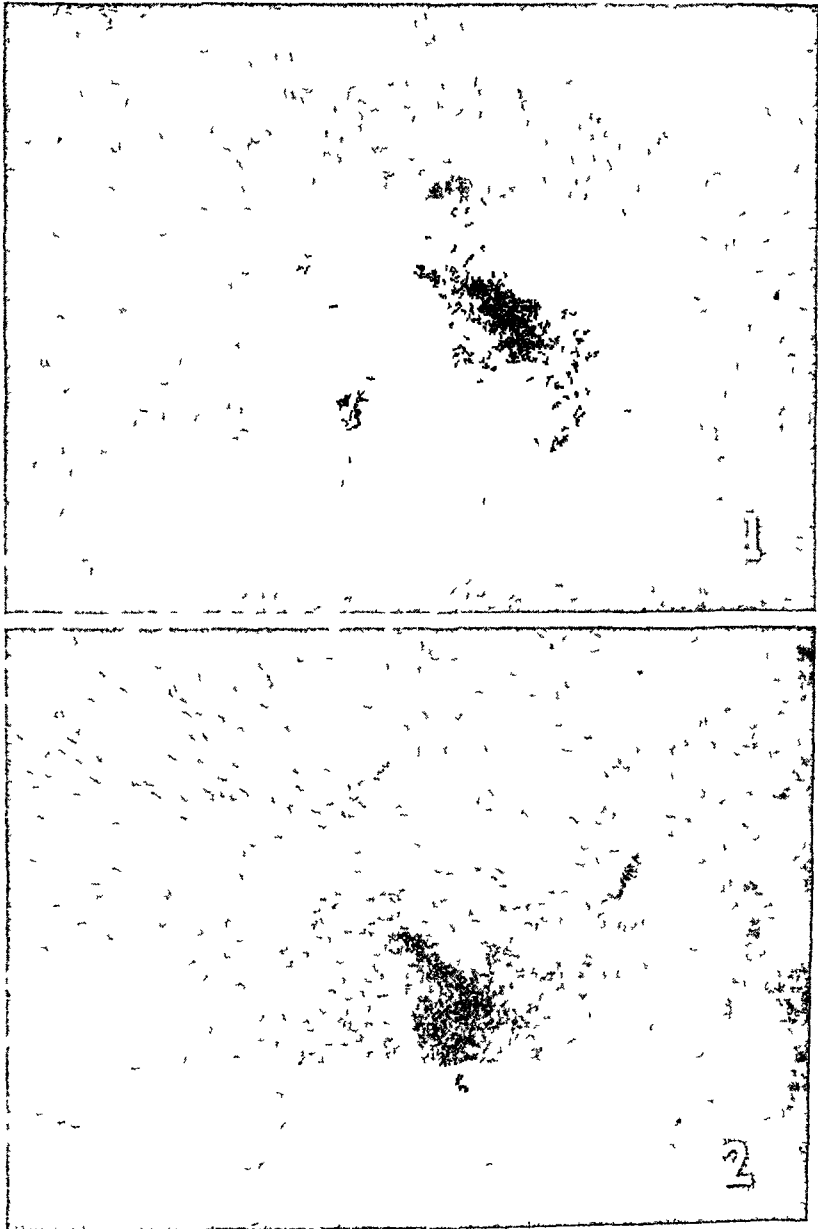


Fig 1—Spreading lesion on the skin of a dicumarolized rabbit eight days after an intracutaneous injection of 0.01 cc of hemolytic streptococcus culture

Fig 2—Localized lesion on the skin of a control rabbit eight days after a similar injection

in 11 of the 15 experiments (73 per cent) In experiment 9, although the treated animal showed little evident local reaction to the infection, death occurred from bacteremia within forty-eight hours

Blood cultures were made on all animals. Seven of 13 that responded to the drug with a prolonged prothrombin time had positive cultures, and 5 of them died with bacteremia in from two to five days. In only 1 control did bacteremia develop, and in this animal death occurred after eight days. Histologic sections of skin from both control and treated animals were prepared and stained for fibrin in 11 of the 15 experiments. In 8 of 10 experiments less fibrin was observed in the skin of dicumarolized animals than in comparable untreated controls. No difference was noted in experiment 10. In only 1 instance was more fibrin observed in the skin of a dicumarolized animal (experiment 14), this animal did not respond to the drug, and the prothrombin time was 5 seconds. Fibrin was not observed in the tissues of animals with a prothrombin time of 3 or more minutes. Conversely, significant amounts of fibrin were observed only in animals in which the prothrombin time was only slightly prolonged.

Animals that did not respond to the drug with a prolonged prothrombin time reacted to the infection in a manner similar to the controls. This would tend to eliminate the action of the drug per se as a causative factor.

SUMMARY

Rabbits treated with dicumarol® usually had an increase of prothrombin time. When they were subsequently infected with an intracutaneous injection of a culture of hemolytic streptococci, the infection spread more extensively than in normal rabbits, and 5 of 13 died with bacteremia. Histologic observations indicate that the lack of fibrin in the tissues of the treated animals is probably a factor in the spread of, in contrast to the localization of, infection in the control animals.

Notes and News

William A Hinton, director of the laboratory of the Boston Dispensary and special consultant of the United States Public Health Service, has been appointed clinical professor of bacteriology and immunology in Harvard Medical School

Frank M Townsend, Temple, Texas, is now associate professor of surgical pathology at the University of Texas Medical Branch, Galveston Dr Townsend will also be consulting surgical pathologist to the hospitals of the University of Texas

Howard T Karsner, former professor of pathology at Western Reserve University, is now adviser to the Surgeon General of the Navy in regard to its medical research program

The Fifth International Congress of Microbiology will be held Aug 17-24, 1950, in Rio de Janeiro, Brazil The secretary of the executive committee is Joaquim Travassos

After a lapse of five years, owing to the war, the Theobald Smith Award in Medical Sciences, established in 1936 by Eli Lilly and Company, will again be given at the annual meeting of the American Academy of Arts and Sciences Fellows of the Academy should submit names of proposed recipients to Dr Gordon K Moe, University of Michigan Medical School, with full information (in triplicate) concerning the personality, the training and the research work of the applicant The award will be \$1,000 and a bronze medal, given for "demonstrated research in the field of the medical sciences, taking into consideration independence of thought and originality" An additional amount of \$150 is available toward traveling expenses The recipient must be less than 35 years of age on January 1 of the year in which the award is to be made, and a citizen of the United States

Academic Press, Inc, announces the publication of *Experimental Cell Research*, sponsored by the International Society for Cell Biology The journal will publish papers dealing with the activity, the structure and the organization of the cell and its subunits, including virus Technical or theoretic papers on experimental cytologic methods will be included Papers may be submitted in English, French or German *Experimental Cell Research* will be edited by Torbjorn Caspersson, Stockholm, Sweden, Honor Fell, Cambridge, England, John Runnstrom, Stockholm, Francis O Schmitt, Cambridge, Mass, Paul Weiss, Chicago, and Ralph W C Wyckoff, Bethesda, Md J F Danielli, London, England, will act as editor of communications for the Society Authors residing in the western hemisphere should send their papers to the editors residing in the United States, those residing in the British Isles should mail theirs to Dr Honor Fell, Cambridge, papers originating in other countries should be forwarded to the Scandinavian editors One volume, consisting of four issues, will be published annually

Books Received

THE CHEMISTRY AND PHYSIOLOGY OF GROWTH By J H Northrop, F O Schmitt, K V Thimann, K Volkers, C B van Niel, E S G Barron, P Weiss, J S Nicholas, C P Rhoads, C N H Long Edited by Arthur K Parpart Cloth Pp 293, with illustrations Price, \$4.50 Princeton, N J Princeton University Press, 1949

The papers were presented at the conference on growth at Princeton University in September 1946

The subject of growth is one of the most important, if not the most important, considerations in biology It is therefore a pleasure and a thought-provoking stimulus to find the subject dealt with so admirably in this book by so many outstanding scientists There is, however, a fundamental question which only one of the contributors considers, and that is—what is growth? One may begin to define growth by calling it a process which spreads before the observer in a display of increasing complexity or of increasing magnitude But having begun in this way the troubles of definition start Increasing complexity is obviously differentiation or something akin to it, increasing magnitude is not universally called growth, as witness, take the swelling of cells or gelation processes Perhaps, as Paul Weiss asseverates in his chapter on "Differential Growth," one cannot study growth but only growing objects Perhaps, with him, we must find the word to be too ambiguous And yet most of us, including nine of the ten contributors, intuitively accept the word unhesitatingly as though we understand what it means We are reluctant to give it up Having warned the reader that there is this philosophic nut to crack, and there are others, too, he is invited to look at the book

The book covers the contributions and integrations of ideas from the subjects of physiologic chemistry, bacteriology, embryology, experimental histology and experimental pathology The synthesis of proteins, cellular metabolism, plant hormones, some nutritional factors and animal hormones go together to make up five of the ten chapters Under experimental histology is included "Molecular Morphology," as revealed by roentgen ray diffraction and electron microscope technics, and tissue culture studies Embryologic development taken as representative of "organization," the study of growth rates of bacterial cultures and the results of investigations having to do with experimentally induced neoplasms round out the book

Of the most direct interest to pathologists is the chapter "Neoplastic Abnormal Growth," by C P Rhoads It would seem to be a fair judgment that in the present state of knowledge one should not expect to find a logically coherent description of the neoplastic process What is available is a collection of views These are covered comprehensively with emphasis on hybridization, mammary tumor inciter and the cytoplasmic control of inherited cellular traits The importance attached to studies of transplantable tumors may be questioned, studies of spontaneous tumor growth are less complicated if more difficult to design For this reason, research on mammary tumor inciter (milk agent) would appear to promise the most interesting information for doctors of medicine

The challenging attitude of Paul Weiss in the discussion of "Differential Growth" will alienate a few pathologists, but many of his arguments on the sterility of morphologic studies limited to one environmental condition of the cells are forceful and convincing. This article contains a large portion of speculation and a number of terms not usually associated with the subject. "Modulation" is not a happy choice of a word to describe the cellular morphologic changes which occur in tissue culture and which are reversed by restoring the cells in an intact organism. Since his main theme is the distinction between reversible differentiation (modulation) and irreversible differentiation it would be simpler, if less elegant, to stick to those terms.

The article by C. B. van Niel, "Kinetics of Growth," contains a dissertation on growth curves in the case of micro-organisms and also two ideas which biochemists generally will not agree with. The first exceptional idea is that when large numbers of individuals are involved (as in cultures of micro-organisms), a statistical treatment is superfluous. Whether this claim is justified will depend on what he means by "treatment." Mathematical statistics have been used to great advantage in the theoretic consideration of large numbers and their behavior. While statistical analysis of data may be superfluous, statistical derivation of concepts may be very useful. The second exceptional idea is that "biochemists in particular have come to recognize that the thermodynamic formulations beg the question which has become paramount in today's biochemistry, viz., that of the mechanism whereby the result is achieved." It would seem the wise course, after the extraordinary success of thermodynamics in chemistry, to withhold judgment. Certainly, the studies of *in vitro* systems described so logically by J. H. Northrop on "The Synthesis of Proteins" and by E. S. G. Barron on "Cellular Metabolism" do not dispense with thermodynamics. The perspective of thermodynamics should be supplementary to the detail and incompleteness of mechanisms.

With respect to the other no less interesting articles the reviewer will point to the "master reaction" in plants proposed by Thimann, will mention the fact that Folkers discusses streptogenin and various derivatives of folic acid in their relationship to growth, and will express his regret that the concept of competitive biologic effects of chemical analogs was practically excluded from the book.

CORRECTION

In the article by Drs. J. H. Cheek and E. E. Muirhead entitled "Bronchial Adenoma Producing an 'Alveolar Cell Carcinoma' Pattern" in the issue of December 1948 (*ARCH. PATH.* 46:529, 1948) the legends for figures 3 and 4 should read as follows:

Fig. 3 (case 1)—The so-called "alveolar cell carcinoma" pattern with papilliferous structure from a metastatic nodule of the opposite lung. Lining of alveolar walls with columnar cells of a mucus-secreting type is evident. This pattern was observed not only as a transition from the adenoma but elsewhere in both lungs.

Fig. 4 (case 2)—The glandular, mucus-secreting make-up of the tumor at its center is demonstrated.

On page 532, in the second paragraph under the sidehead "Autopsy," "fig. 3" should be deleted and "fig. 4" should be fig. 3.

RELAPSING FEBRILE NODULAR NONSUPPURATIVE PANNICULITIS

A Report of a Case with a Review of the Literature

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AND

SAMUEL G PLICE, M D

CHICAGO

THIS IS a disease aptly described by its name. It is characterized by recurring bouts of fever associated with the appearance of varying-sized subcutaneous nodules that are usually erythematous and may be painless or slightly tender. The nodules develop most commonly on the thighs and the arms, often on the abdomen and the back and occasionally on the lower parts of the legs. Over a period of from weeks to months the lesions regress, leaving shallow or atrophic areas where fatty tissue of the panniculus adiposus has locally disappeared, with the skin becoming subsequently attached to deeper structures. Sometime during the course there are subjective symptoms of varying intensity, including malaise, fatigue, fever, chills, generalized pains, nausea and headaches.

Pfeifer¹ made the first report in 1892 and pointed out that similar changes could be produced in the fat tissue by artificial means. In 1916 Gilchrist and Ketron² reported the second case. Weber,³ in 1925 and Christian,⁴ in 1928, in reporting the third and fourth cases, respectively, added the descriptive words by which this disease entity is still known. There were several more or less typical cases reported during the next fifteen years, until 1943 when Miller and Kritzler⁵ reviewed 26 cases in the literature and reported 1 case. The patient was a 34 year old Jewish woman who had an unusually severe illness and died with acute lesions of relapsing febrile nodular nonsuppurative panniculitis. The autopsy was noncontributory to our understanding of the disease.

1 Pfeifer, V. *Deutsches Arch f klin Med* **50** 438, 1892

2 Gilchrist T C, and Ketron, L W. *Bull Johns Hopkins Hosp* **27** 291, 1916

3 Weber, F P. *Brit J Dermat* **37** 301, 1925

4 Christian, H A. *Arch Int Med* **42** 338, 1928

5 Miller, I L, and Kritzler, R A. *Arch Dermat & Syph* **47** 82, 1943

Since then reports of 8 additional cases have appeared Larkin, de Sanctis and Margulis⁶ reviewed the subject and added a case The patient was a 23 month old white boy with a history of nodules on his ankles, which regressed into atrophic areas while he was in the hospital Biopsy of these areas gave results compatible with this disease

Another case was reported by Spain and Foley⁷ The patient, a 51 year old Irish man, entered the hospital with uremia On the second day, subcutaneous nodules developed The patient died of uremia, and at autopsy these nodules revealed a characteristic appearance, moreover, nodules were found not only in the subcutaneous fat but in the mesenteric and omental and pretracheal fat

Friedman⁸ reported another case with autopsy A 23 year old woman had recurrent crops of painful red spots and elevated nodules, some of which ulcerated There were associated fever, generalized aching, cough, fatigue and splenomegaly with prominent leukopenia A culture of *Staphylococcus aureus* was obtained from the heart blood at autopsy, and the patient was believed to have died of staphylococcal septicopyemia No changes were found in the visceral adipose tissue

Arnold⁹ reported a case A 27 year old Caucasian woman had moderately tender, slightly raised, deep-seated nodules on the anterior part of the left thigh Sulfadiazine and sulfathiazole were given without benefit, but sulfapyridine seemed specific, five relapses occurring when administration of this drug was discontinued and five remissions on readministration The histologic picture of the biopsy tissue was typical

Ives¹⁰ reported a case in which a 53 year old white man showed a disease which clinically was compatible with the diagnosis of Weber-Christian disease, but in which no biopsy was made to confirm the diagnosis

The case of a 23 year old soldier who had warm, erythematous tender nodules, fever and malaise was reported by Zee¹¹ Biopsy of the lesions revealed a typical picture Penicillin therapy was tried, and a clinical remission followed, but whether this was spontaneous or due to the effect of the therapy was left undetermined

A report of a case with autopsy was made by Ungar¹² A 37 year old woman died from suppurative peritonitis Tissues were taken both before death and at the postmortem examination, so that various stages

6 Larkin, V de P, de Sanctis, A G, and Margulis, A E *Am J Dis Child* **67** 120, 1944

7 Spain, D M, and Foley, J M *Am J Path* **20** 783, 1944

8 Friedman, N B *Arch Path* **39** 42, 1945

9 Arnold, J L *Arch Dermat & Syph* **51** 94, 1945

10 Ives, G J *Missouri M A* **42** 409, 1945

11 Zee, M L *J A M A* **130** 1219, 1946

12 Ungar, H *J Path & Bact* **58** 175, 1946

in the development of the nodules were observed and the various microscopic changes discussed. Characteristic lesions were present throughout the adipose tissue.

Mostofi and Engleman¹³ reported a case of a 39 year old Filipino soldier who had recurrent symptoms for seven months with recurring nodules that regressed and left no scars. The patient died, and autopsy revealed widespread involvement of the fat tissue. Typical lesions were found in the subcutaneous, epicardial, peripancreatic, periadrenal, perirenal and mesenteric fat.

An interesting report of a similar condition in rabbits was made by Duran-Reynals¹⁴. He discussed the analogies with the disease in man.

REPORT OF CASE

G. H., a 44 year old Negro man, was admitted to Cook County Hospital, Chicago, on July 21, 1947, complaining of small "bumps" which had been present beneath his skin for two months. In the two weeks preceding entry he had lost about 10 pounds (4.5 Kg.) and suffered from marked weakness and fatigability. In addition, he had felt feverish much of the time and occasionally had slight chills, followed by sweating. During the past few days he had noticed that the masses were receding in size. The history revealed that the patient had experienced three episodes of similar nodules and symptoms, the first eight years ago. Each episode had been ushered in with the appearance of nodules, which continued to increase in size for about two months and then to wane, taking approximately the same length of time to disappear. With the latter phase there had always been malaise, weakness, loss of weight and fever until the nodules disappeared. He had never received treatment. A history of syphilis and gonorrhea fifteen to twenty years before and also a history of malaria in 1934 while he was living along the Gulf of Mexico was obtained.

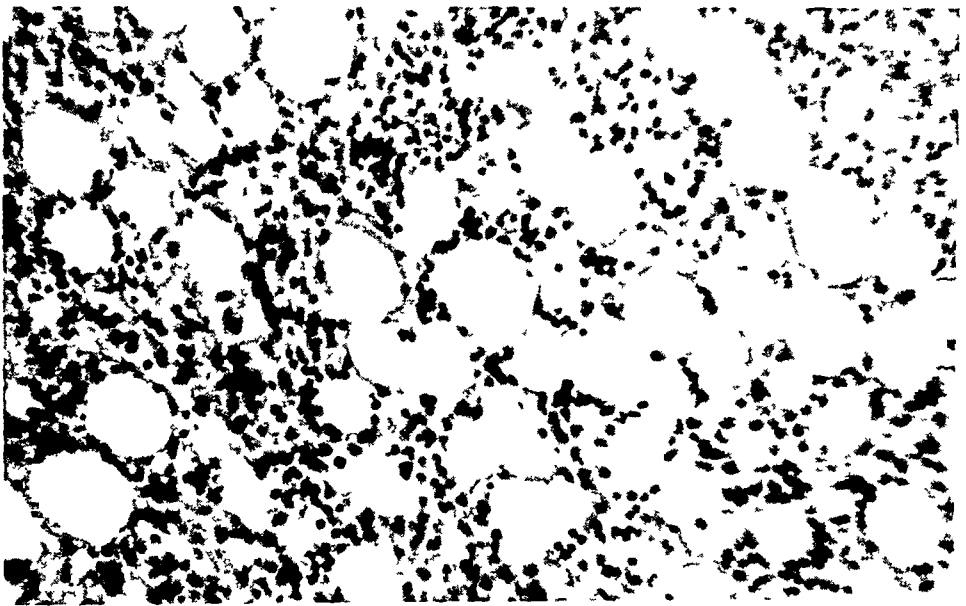
From the time of admission he had a continual low grade fever, the temperature reaching 101.4 F. Scattered over the arms, abdomen, chest, thighs and to a lesser degree the back were multiple nodules ranging from 1 to 3 cm. in size. These were firm in consistency and nontender to touch, a few were fixed to the skin, but most of them were movable. Lymph glands of the axillae and groins were enlarged. The liver was palpable 3 to 4 cm. subcostally, the spleen was not palpable. Physical examination gave otherwise normal results.

The initial white blood cell count was 1,800, and repeated counts never exceeded 3,950. Of these, 36 to 45 per cent were lymphocytes. The red blood cell count was 2,830,000 and 3,000,000 on two occasions, respectively. There was a moderate amount of anisocytosis, and occasional target cells and toxic lymphocytes were seen on a peripheral blood smear. The blood nonprotein nitrogen was 26 mg. per hundred cubic centimeters. The acid phosphatase was 0. Inorganic phosphorus was 5.1 mg. per hundred cubic centimeters. The total protein was 7.6 Gm. per hundred cubic centimeters, with a reversal of the albumin-globulin ratio (3.1:4.5). The Kahn test of the blood was negative. Urinalysis gave normal results repeatedly. Roentgen examination of the chest revealed no hilar or parenchymal abnormalities and a heart that was within normal limits. Roentgen examination showed the hands to be normal.

13 Mostofi, F. K., and Engleman, E. *Arch. Path.* **43**: 417, 1947.

14 Duran-Reynals, F. *Yale J. Biol. & Med.* **18**: 583, 1946.

A biopsy of one of the nodes over the thigh revealed that the collagen connective tissue of the papillary and reticular layers of the dermis was dense, with slight edematous changes of the papillary layer. The blood vessels lying within that layer were surrounded by small numbers of lymphocytes. The loose, edematous connective tissue about some of the hair follicles and sweat glands was infiltrated by lymphocytes and occasional large mononuclear cells. Within the subcutaneous fat tissue there was a poorly circumscribed nodule which measured 10 by 4 by 3 mm. The nodule itself was composed of rather dense collagenous connective tissue, fat tissue and nests of simple sweat glands. The connective tissue about these sweat glands showed slightly bluish-staining cytoplasm suggestive of pseudomyxomatous degeneration of the connective tissue fibers, and was infiltrated by lymphocytes. The fatty tissue, on the other hand, showed an



Microscopic appearance of the lesion in the fat tissue of the skin

extensive cellular infiltrate, including lymphocytes, plasma cells, large mononuclear cells and an occasional polymorphonuclear leukocyte, widely separating the individual fat cells and compressing some of them. In the fibrous connective tissue there were cellular infiltrates similar to that seen in the fat tissue, particularly about the blood vessels. The endothelium of these blood vessels was slightly swollen.

The patient remained in the hospital for eighteen days, during which time the nodules progressively decreased in size. During the first week of hospitalization the patient continued to suffer from weakness, fatigue, chills and fever, but during the latter part of his stay these symptoms gradually diminished in intensity. He was released on August 7, and at that time he was feeling much improved and the nodules were barely palpable. The patient returned three weeks later to the outpatient clinic feeling entirely normal and without any evidence of the former nodules or of their previous locations.

COMMENT

To date 35 cases of relapsing febrile nodular nonsuppurative panniculitis have been reported. Of the patients, 25 were women and girls and 10 males. Of these patients, 6 have died from varied causes (only 1 definitely as a result of this disease), and lesions were examined at autopsy in 5 instances, thus adding considerably to knowledge of the disease. Beforehand it had been thought that the lesions were limited to the panniculus adiposus, but in 3 of the postmortem examinations¹⁵ the inflammatory nodules were found not only in the subcutaneous fat but, in varying intensity, in the fat tissue of the abdomen and thorax.

Histologically, the fundamental feature is a cellular infiltration of the subcutaneous tissue, followed by edema and phagocytosis of the fat elements by macrophages, with a fibrous reaction occurring in later stages. A new concept of the histologic progression has been gained from 3 cases in which nodules in various stages of development or regression were obtained and examined. From these studies there have been described three main stages of the disease. First there is the early stage, in which the nodules have just appeared and are barely palpable. These reveal a moderate degree of infiltration, predominantly of leukocytes, between the fat cells. Then there is the large, well developed nodule, which probably represents the stage which has been most commonly described. In it the normal fat tissue lobule is replaced by many fat-laden macrophages plus lymphocytes, polymorphonuclear leukocytes and others. The third or late, atrophic stage is relatively acellular. Only a few lymphocytes and an occasional giant cell remain in the white fibrous tissue.

The cause of this disease is still obscure. Various chemical, thermal, infectious and mechanical injuries have been suggested but never proved to be causative. The use of iodides and bromides has preceded the onset of this disease in some instances and therefore has been regarded as a possible factor. Other factors, such as the injection of insulin or of hypertonic dextrose and sodium chloride solution and avitaminosis, have also been mentioned. In the case reported here there was nothing in the past history which gave any clue to the cause.

Some believe this to be an infectious disease. In a fair number of the cases foci of infection have been found, most often in dental cavities and tonsils. The fact that nodules have been widely dispersed in fatty tissue might suggest a blood-borne agent. Repeated attempts to isolate bacteria from the lesions by smears and cultures, however, have been made without success. This made some consider the possibility of a virus infection. The character of the lesion, with destruction of fat and muscle, followed by infiltration and fibrosis, makes it similar to

¹⁵ Spain and Foley¹¹ Ungar¹² Mostofi and Engleman¹³

many viral diseases. Others have entertained the possibility of its being an evidence of bacterial allergy. Arnold⁹ made an interesting comparison with dermatitis herpetiformis, which also is a chronic relapsing disease, often aggravated by iodides and bromides, and which responds better to sulfapyridine than to other sulfonamides.

No treatment of this disease has been established as successful. In 1 case in which sulfadiazine and sulfathiazole had failed to produce any effect, the response to sulfapyridine made this drug seem specific, five relapses occurring on stopping the drug and five remissions on readministration. Penicillin was used in another instance,¹¹ but the part it played in the course of the disease is in question. In our case there were no acute symptoms and no treatment had been given, for the patient had improved by the time the diagnosis was certain.

SUMMARY

The case reported here, the thirty-sixth, agrees in the important clinical and histologic characteristics described in the literature for relapsing febrile nodular nonsuppurative panniculitis. The distribution of the nodules was typical, and the feature of recurrences was well demonstrated, the patient having entered the hospital in the fourth relapse. Low grade fever was present, but never the high, spiking temperature reported by some. Leukopenia was found in this patient as has been noted in about one half of the cases. There were no pitted areas in the skin following healing, but this is understandable in a thin panniculus such as this patient had, in which the depression would be minimal and easily overlooked. By virtue of the repeated spontaneous remissions in this patient, real doubt was cast on the therapeutic value of some medications heretofore reported.

ONKOCYTIC ADENOMA OF THE SALIVARY GLANDS

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NEW YORK

ONKOCYTES, first named by Hamperl,¹ are cells occurring in the ducts and acini of the salivary glands and in other locations. These cells had previously been studied by others. The word "onkocytoma" was introduced by Jaffe² for the tumor usually called "papillary cystadenoma lymphomatosum." Skorpil³ recommended that this use of the term be dropped and that "onkocytoma" be used for the onkocytic tubular adenoma of the salivary glands. He further suggested "onkocytic adenoma" as a possible term for this type of tumor. Ackerman⁴ used the term "onkocytoma" in the sense recommended by Skorpil and, like Skorpil, stated that it should be restricted to tumors located in the salivary glands.

Hamperl derived the name "onkocyte" from the Greek word *ογκοῦσθαι*, meaning "increase in bulk." It is to be noted that this Greek word is related to the Greek word *ογκος*, meaning "tumor" or "bulk," from which the English word "oncology" is derived. He found these cells in the ducts and acini of salivary glands and the serous, mixed and mucous glands of the tongue, the pharynx, the esophagus and the trachea. These cells are not found in these locations in persons under the age of 20 years. They become more prevalent in older age groups and can be found in practically every person over 60 years of age. Hamperl also felt that onkocytes should include similar cells appearing on rare occasions among the acinous cells and lining duct cells of the pancreas. He did not feel that their presence in the islands of Langerhans had been definitely established. He included the eosinophilic (Welsh) cells of the parathyroid glands, the Askanazy cells of the thyroid gland, the eosinophilic cells of Rathke's pouch, certain eosinophilic cells of the anterior lobe, the posterior lobe and the stalk of the pituitary gland and islands of characteristic cells of the lining

From the Department of Pathology, New York Post-Graduate Medical School and Hospital

1 Hamperl, H. Ztschr f mikr-anat Forsch **27** 1, 1931, Virchows Arch f path Anat **298** 327, 1936

2 Jaffe, R H. Am J Cancer **16** 1415, 1932

3 Skorpil, F. Virchows Arch f path Anat **306** 714, 1940

4 Ackerman, L V. Arch Path **36** 508, 1943

epithelium of the uterine tube Hamperl⁵ observed onkocyte-like cells in the lining epithelium of a few of the seminal tubules of an atrophic testis and in small nodules of parenchymal cells comprising portions of each of several hepatic cords of a cirrhotic liver He did not feel that it had been established yet that onkocytes occur in the testis and the liver, because they had been observed in these two organs in only 1 case each Hamperl⁵ found cells somewhat resembling onkocytes among the mucous cells of the cardiac glands of the stomach in 2 cases These cells differed from onkocytes in that the cytoplasm was more homogeneous and took a yellowish red rather than a red color with erythrosin-safranine

Stout⁶ noted onkocytes in the acini and ducts of the mucous glands of the bronchi I have seen such cells in the ducts of the mucous glands in the vocal cords and the tonsillar capsule Boeck and Schlagenhauff⁷ found them in the lacrimal gland Boeck⁸ found them in a tumor of the lacrimal sac, and Radnot⁹ has also seen them in the lacrimal sac LaManna¹⁰ described connective tissue cells with this appearance but probably was referring to macrophages with a granular eosinophilic cytoplasm These occurred in the regions of breast where tissues had been removed for determination of carcinoma Allegranza¹¹ expressed the belief that these cells eventually will be recognized in all tissues It is to be noted that they have been observed only in adults or in pathologic tissues The Welsh cells of the parathyroid glands are somewhat of an exception They do not appear until late childhood but have been found as early as the seventh year They probably have no secretory function The onkocytic cells of the anterior lobe of the pituitary gland differ in appearance from the functional eosinophilic cells, being larger darker and otherwise resembling onkocytes of other organs

This heterogenous group of cells has been included under one name because of similarities in appearance They resemble the cells of the tissue in which they are found but are larger and have a cytoplasm which is filled with fine, brightly eosinophilic granules or is itself brightly eosinophilic and finely honeycombed Hamperl¹ expressed the belief that the honeycombing is a transitional stage in the development of the fully differentiated granular cytoplasm from the normal cell The nucleus is often pushed toward the lumen or is in the center of the cell

5 Hamperl, H Virchows Arch f path Anat **296** 82, 1936

6 Stout, A P Arch Path **35** 803, 1943

7 Boeck, J, and Schlagenhauff, K Ztschr f Augenh **94** 244, 1938

8 Boeck, J Ber u d deutsch ophth Gesellsch **53** 299, 1940

9 Radnot, M Ophthalmologica **101** 96 1941

10 LaManna, S Arch per le sc med **66** 191, 1938

11 Allegranza, A Ann di biol norm e pat **1** 242, 1946

It resembles those of the surrounding cells or stains slightly darker and may appear smaller. Hamperl¹ expressed the belief that the apparent smaller size is due to a covering of the edges of the nucleus by the many fine cytoplasmic granules. Hamperl and others have stated that these cells divide by amitotic division. No normal mitoses have been seen except in the onkocytoma reported by Ackerman.¹ Hamperl expressed the opinion that these cells are not degenerative cells but represent a "further differentiation" or an "aging process." Allegranza¹¹ stated that the granules consist of proteins, sometimes linked with lipids. He expressed the belief that the honeycombed appearance of some of these cells is the result of the dissolving out of the fat in the preparation of paraffin sections. These granules sometimes take a faint fat stain. Skoipil³ expressed the belief that in the case of onkocytoma which he reported this change was developing in the neoplastic cells, the onkocytic change occurring after the tumor's origin. Hamperl¹ also suggested the possibility of this type of change. The finding of smaller, denser-staining cells reported by several authors and the transition leading to disintegrating cells seen in my case suggest that this may be akin to a retrogressive change. It differs from the usual degenerations in that it is an apparently permanent, nonreversible process, is found to have been transmitted to daughter cells, following amitotic division, is usually connected with a loss of the ability to undergo normal mitosis and is related to the aging of the individual or to a pathologic process. Allegranza¹¹ has taken a somewhat similar view. None of the onkocytes are known to possess any function other than a mechanical one, such as lining ducts, although mucus was seen in some of the ducts of the tumor studied by me.

In view of the wide variation in types of tissue showing onkocytes, the retaining by onkocytes of some features of their original morphologic aspect and the observation presented in the foregoing paragraph as made by Skoipil, I feel that this phenomenon should be regarded as a change in the cells and not a change to a specific type of cell. Since use of the word "onkocyte" would imply that these are a specific type of cell, it would be preferable to speak of "onkocytic salivary duct cells" (for instance) rather than "onkocytes of the salivary ducts" and the term "onkocytic adenoma" should be used rather than "onkocytoma." The term "onkocytic adenoma" is also preferable because "onkocytoma" does not carry any implications as to the structure or the cancerous nature of the tumor. Although the use of "onkocytoma" has been opposed for papillary cystadenoma lymphomatosum¹² that use is still widespread and causes confusion between these two tumor types. I feel that the term "onkocytic adenoma" should not be restricted to tumors of the salivary glands.

12 Hamperl¹ Ackerman⁴

Not only are onkocytes found in onkocytic adenoma but they are also the epithelial cell type in papillary cystadenoma lymphomatosum in many cases and in simple salivary gland cyst and mixed tumor of the salivary glands in some cases¹ I have seen a small onkocytic cyst of the pharynx and have seen onkocytic areas in carcinoma of the parotid gland In view of the occurrence of onkocytic cells and areas in other types of salivary tumors, a number of blocks should be studied in any case suspected of being onkocytic adenoma to exclude this possibility,

Cases of Onkocytic Adenoma

Author	Age, Yr	Sex	Duration	Site	Shape and Size	Consistency	Color	Attachment to Skin	Follow up
McFarland ^{13, 14}	71	M	3 yr	Right parotid region	4.5 × 4 × 2 cm	Soft solid	Dark brownish red	None	Recurrence 2 yr later, died 6 mo later of other causes
Blair and Oieh ¹⁵ Huckel ¹⁷	61	M	8 yr	Parotid region	Plum size	Soft	Gray red		
Steinhardt ¹⁹	67	M		Right parotid region				Well encapsulated	
Ahlbom ²⁰	59	M	4 mo	Hard palate, fixed to bone	1.5 × 2 cm				No recurrence after 3 yr, 8 mo
Gruenfeld and Jorstad ¹⁶	68	F	3 yr	Right parotid region	Globular, 3 × 4 cm	Firm and elastic	Brownish red	None, encapsulated	
Skorpil ³	83	F		Parotid region	Size of child's fist		Gray brown	Well encapsulated	No recurrence after 2 yr
McFarland ¹¹	74	F	Many years	Angle of left jaw	Pea size				
Ackerman ⁴	76	M	6 mo	Left parotid region	2 cm	Firm	Brownish red	None	No recurrence in 8 mo
Lloyd ¹⁸	59	F		Preauricular region	3 cm ?				
Author's case	53	M	18 mo	Left parotid region	Round, 2.5 × 2.8 × 1.8 cm	Moderately firm	Brownish gray	None	

although the diagnosis of "onkocytic adenoma" does not require that all cells be onkocytes (See later comment on Skorpil's and Ahlbom's cases)

Ten cases of onkocytic adenoma of the salivary glands have been described (table) Five additional cases of tumors have been considered by some authors as cases of onkocytic adenoma

REPORT OF A CASE

A 53 year old white man seen at the New York Post-Graduate Medical School and Hospital had noticed a freely movable swelling of the left side of the face and angle of the jaw for one and one-half years but had had no distress, pain or tenderness He was not sure whether there had been any recent growth

The regional lymph nodes appeared normal. At operation the tumor was found embedded in the parotid gland. A branch of the facial nerve passed through it. The specimen was rounded and measured 2.5 by 2.2 by 1.8 cm. It was entirely encapsulated. The entire tumor was blocked for section.

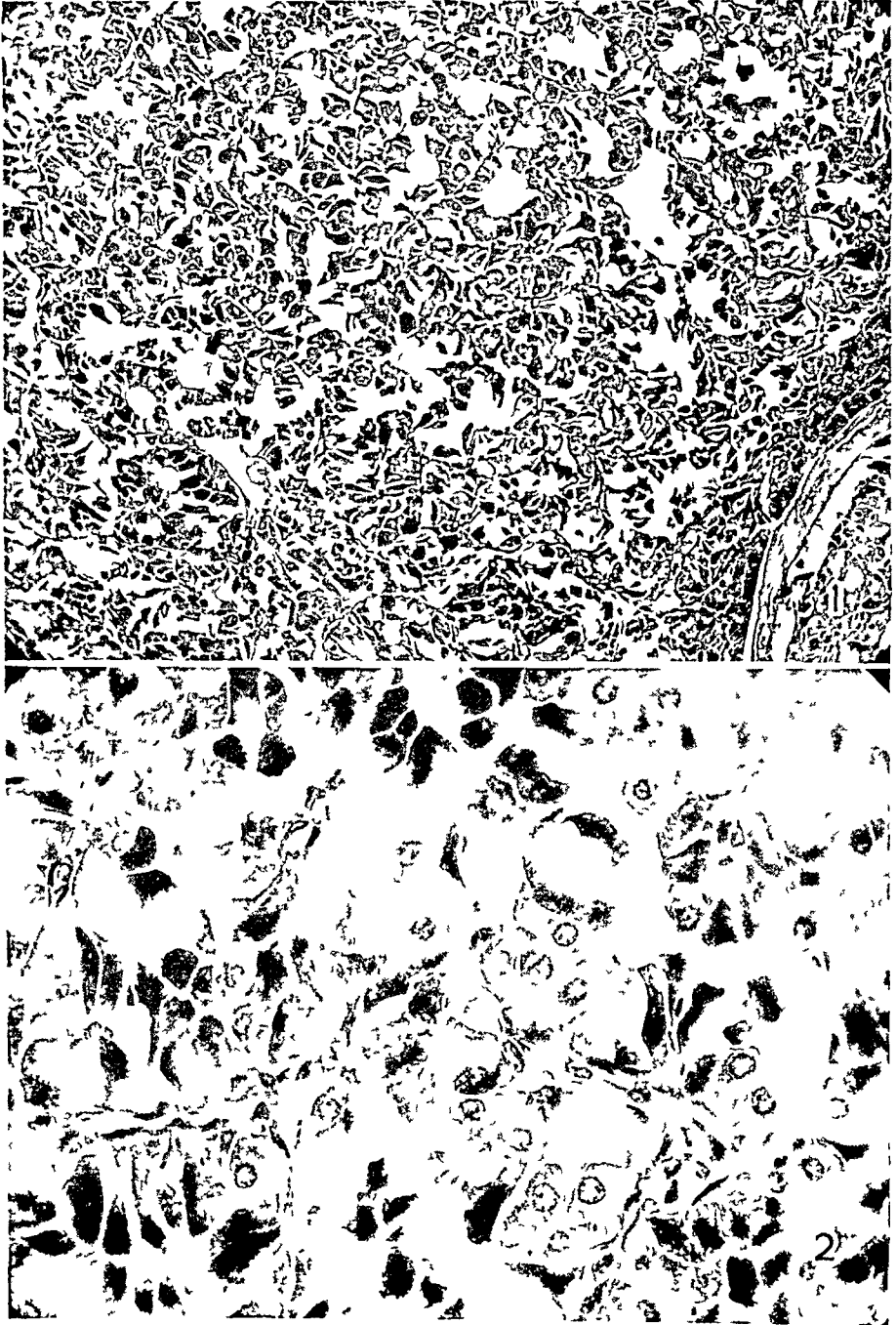


Fig 1—Onkocytic tumor. The general structure of the tumor is illustrated. Several ductlike structures are seen. $\times 100$

Fig 2—Thin fibrous septums separating masses of onkocytic cells and the darker, sclerotic cells, which are usually smaller. $\times 400$

Microscopically, the tumor was well encapsulated. Attached to the capsule was adipose and fibrous tissue containing in some areas groups of ducts suggesting atrophic remnants of the parotid gland. Many of these ducts were made up of

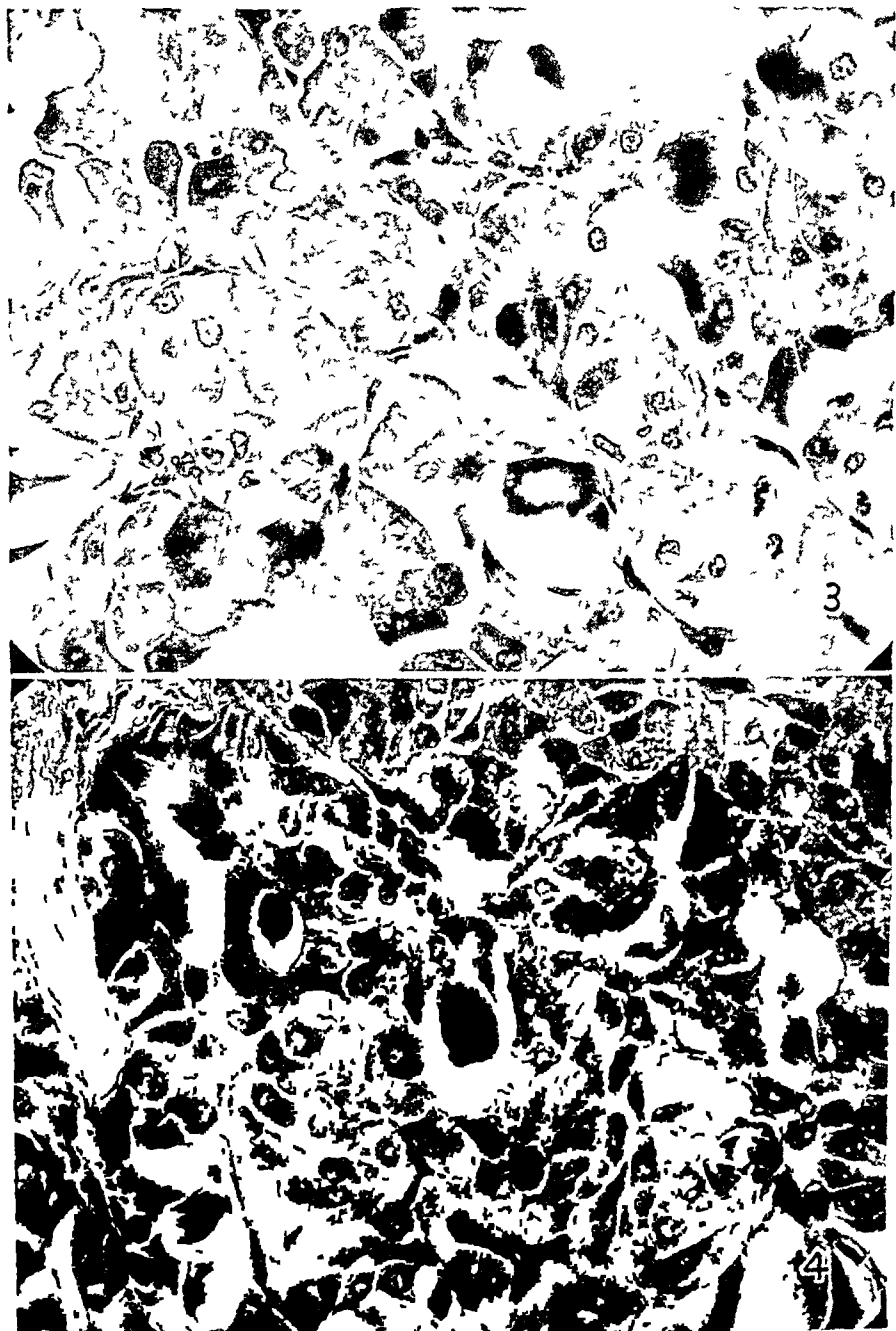


Fig 3—Onkocytic and sclerotic cells. One mass of secretion is seen. $\times 400$

Fig 4—Ductlike structures containing an opaque material, which stains with the mucicarmine stain. $\times 400$

onkocytes. There were areas of hemorrhage and of deposition of hemosiderin. One area showed compressed parotid tissue with degenerative changes in the acinous

cells. Small masses of tumor cells were seen in the capsule, but they appeared to be entrapped in it rather than invading it. One collection of approximately 18 cells was seen in a vessel. Their presence there was interpreted as an artefact. The tumor was divided into lobules of various sizes and shapes by hyalinized fibrous tissue septums of differing width. Narrower septums subdivided many of the lobules. The cells were from 7 to 22 microns in diameter. They were of irregular polygonal shape and formed fairly solid masses, which not infrequently contained groups of 8 to 17 high cuboidal or columnar cells forming ductlike structures. A few of these contained material which took a pale or moderate mucicarmine stain, but no mucus was seen intracellularly or outside these structures. A few of them were enlarged and showed flattened, compressed cells. The cytoplasm of the cells was made up entirely of fine granules which took a bright stain with eosin and an intense dark, opaque red with Masson's trichrome stain. The cells differed in the abundance of the granules. None of the cells had a foamy cytoplasm. Most of the nuclei were moderately vesicular and resembled those of the normal parotid gland. Many, however, were smaller and stained more heavily. These cells did not take the sudan IV stain. Scattered singly among the other cells and occurring in clusters were smaller ones in which the cytoplasm was darker and more homogeneous. The nuclei were also smaller and darker. That this was a degenerative process was suggested by the fact that the cells showed transitions in appearance leading to clusters of cells with disintegrating cytoplasm and pyknotic nuclei.

COMMENT

The first case of McFarland¹³ was described in 1927 and referred to briefly in 1942.¹⁴ McFarland's second case was not described anatomically, but in view of his wide experience, should be accepted. It is interesting, in view of Allegranza's¹¹ designation of Hürthle cell tumors as "onkocytic" tumors of the thyroid gland, that McFarland calls these tumors "Hürthle cell tumors of the parotid" and only as a subheading calls them "onchocytomas."

I have not been able to obtain the report of Blair and Olch,¹⁵ but their case was accepted by Gruenfeld and Jorstad.¹⁶ Ackerman⁴ did not include it in his list.

The cases of Huckel¹⁷ were not accepted by Gruenfeld and Jorstad and were not included in Ackerman's list. His first case only is accepted by Lloyd¹⁸ and by Skorpil,³ who studied the slides. In view of Skorpil's careful studies of this type of tumor, I feel that his judgment should be accepted. His paper shows a photomicrograph of Huckel's tumor that is typical in appearance. Hamperl accepted Huckel's 3 cases but with less careful study than Skorpil gave them.

13 McFarland, J. *Am J M Sc* **174** 362, 1927

14 McFarland, J. *Am J M Sc* **203** 502, 1942

15 Blair, V. R., and Olch, I. Y., cited by Gruenfeld and Jorstad¹⁶

16 Gruenfeld, G. E., and Jorstad, L. H. *Am J Cancer* **26** 571, 1936

17 Huckel, R. *Ber u d deutsch path Gesellsch* **25** 342, 1930

18 Lloyd, O. C. *J Path & Bact* **56** 699, 1946

Steinhardt's¹⁹ case was rejected by Skorpil but is accepted by Ackerman⁴ and Hamperl.¹ His tumor had a characteristic gross appearance and microscopically was lobulated and had cells with a brightly eosinophilic cytoplasm. The centers of the lobules were made up of cells which were about the size of liver cells and had a granular cytoplasm. At the borders of the lobules the cells were larger and of loose structure with a netlike cytoplasm. The foregoing description is much like that of most of the other onkocytes reported. He also spoke of the cytoplasm as being "fibrillar," but when this is interpreted in the light of the rest of the description, it does not appear to indicate that the cells were strikingly different from other onkocytes. His description of the nuclei is fairly typical for onkocytes. The photomicrograph cannot be recognized as that of onkocytic adenoma, but the plate is dark. It shows cells in parallel rows. Steinhardt spoke of the cells of his tumor as being "transitional forms," such as Hamperl described in tracing the origin of the onkocytes from the cells of the salivary gland ducts, but his description seems typical enough to me to warrant inclusion of this tumor in the group designated as onkocytic adenoma.

Ahlbom's²⁰ description is too brief for evaluation, but the illustration is typical and the case is accepted by Ackerman⁴ and by Skorpil.³ Hamperl studied the slides and accepted this case, although he stated that some fields showed cells with honeycombed basophilic cytoplasm.

The remaining cases are typical (table, cases of Ackerman,⁴ Gruenfeld and Jorstad¹⁶ and Lloyd¹⁸). Skorpil's case is interesting in that it shows a poorly outlined area in which the cells are smaller and have a weakly basophilic cytoplasm. Typical onkocytes are scattered in this area, and the borders show a gradual transition of cell type to the onkocytes of surrounding areas. This structure led Skorpil to suggest that the cells changed to onkocytes after the formation of the adenoma.

CLINICAL FEATURES

The onkocytic adenoma has always behaved as a benign tumor, although in the first case of McFarland¹⁴ it recurred after twelve years. In view of the characteristics of this type of tumor I feel that the second growth could have resulted from an additional focus of origin. Pre-operatively the tumor is usually considered to be a mixed tumor.

CASES WHICH ARE NOT ACCEPTED AS INSTANCES OF ONKOCYTIC ADENOMA BY THE AUTHOR

Stohr and Risak²¹ reported 2 cases which Ackerman⁴ included in his list. Skorpil³ did not make a definite statement but apparently did

19 Steinhardt, G. *Virchows Arch f path Anat* **289** 624, 1933

20 Ahlbom, H. E. *Acta radiol (supp)* **23** 96, 1938

21 Stohr, F., and Risak, E. *Arch f klin Chir* **143** 609, 1926

not consider them as cases of onkocytic adenoma. Hamperl did not mention these cases. The 2 cases are accepted by Gruenfeld and Jorstad¹⁶. The first one (Pr Nn 618/1911) is called a case of tubular adenoma, but no histologic description is given. The authors did not illustrate this case. The second case (Pr Nn 301/1925) was one of an encapsulated lobular tumor which microscopically had an acinous structure, with cells showing a marked similarity to the parenchymal cells of the normal serous gland. Some areas showed a granular cytoplasm. This tumor contained cavernous vascular structures. The photomicrograph is blurred. The cells in it look small and not too much like onkocytes. The authors did not mention the staining reaction of the cells. It is my feeling that no tumor should be classified as an onkocytoma unless its cells are known to take a bright eosinophilic stain, in addition to other characteristics, so I have not included these 2 cases.

Franssen's²² case was accepted by Lloyd¹⁸ but was not included in Ackerman's⁴ list and Skorpil,³ who had seen the slides, did not accept it, stating that its cells had a clear cytoplasm.

Lloyd stated that Leroux and Leroux-Roberts²³ reproduced a photomicrograph (their fig. 2) like Lloyd's onkocytic adenoma. It is true that Leroux and Leroux-Roberts showed several photomicrographs that suggest onkocytic adenoma, but their paper is a classification of the epithelial tissues of mixed tumors, and they did not give a complete description of their material, thus leaving the possibility open that all of these might be onkocytic areas of some other type of lesion.

ONKOCYTIC TUMORS OF OTHER ORGANS

Hamperl held that the eosinophilic tumors of the parathyroid glands, of which Castleman and Mallory²⁴ have reviewed 160 cases, is onkocytic adenoma. Hamperl and Allegranza held that the Askanazy tumor of the thyroid gland is onkocytic adenoma. Tumors of the latter name are called Hurthle cell tumors in this country. Most European authorities reject the name "Hurthle cell tumors" because they feel that the cells which Hurthle described are not the ones found in these tumors. The cells described by Hurthle were found in dogs in the interfollicle tissue of the thyroid gland. Similar interfollicle cells have not been found in man. The similar-appearing Askanazy cells do not occur in children but are found in adults and in pathologic conditions. They are found as epithelial cells lining follicles. Priesel²⁵ reported a tumor of the

22 Franssen, R. *Centralbl f allg Path u path Anat* **56** 113, 1932-1933.

23 Leroux and Leroux-Roberts. *Bull Assoc franç l'étude cancer* **23** 304, 1934.

24 Castleman, B., and Mallory, T. B. *Am J Path* **11** 1, 1935.

25 Priesel, R. *Virchows Arch f path Anat* **267** 354, 1928.

pancreas which has been accepted as onkocytic adenoma by many authors, including Hamperl, who saw the slides. A small number of tumors diagnosed as onkocytic adenoma were accepted by Hamperl as having occurred in the posterior lobe, the anterior lobe and the pedicle, respectively, of the pituitary gland. In some instances adenoma of the bronchus was made of onkocytes.⁶ Zippel²⁶ reported 1 case in which adenoma of the adrenal gland, and 1 case in which adenoma of the renal cortex, was made up of onkocytes. Boeck⁸ reported a case of onkocytic adenoma of the wall of the lacrimal sac and Radnoty²⁷ a case of onkocytic cyst of the same organ.

SUMMARY

Ten cases of onkocytic adenoma of the salivary glands and related glands of the hard palate have been reviewed and an additional one has been reported.

Certain tumors of the thyroid, parathyroid, pituitary and adrenal glands, the pancreas, the lacrimal sac and the kidneys have been reported to be onkocytic adenoma.

The possibility of the onkocytic change being a retrogressive one of unique type should not be discarded. The change should not be regarded as the development of a specific type of cell. The term 'onkocytic adenoma' is preferable to "onkocytoma."

²⁶ Zippel, cited by Allegranza¹¹

²⁷ Radnoty M. *Ophthalmologica* **113** 270, 1947

EFFECT OF PROLONGED INTRAVENOUS ADMINISTRATION OF DEXTROSE ON BETA CELLS OF ISLETS OF LANGERHANS

S STEVEN BARRON, M D

AND

DAVID STATE, M D

MINNEAPOLIS

THIS is a preliminary report on studies being conducted to determine the function of the beta granules of the islets of Langerhans, with special emphasis on their role in diabetes mellitus. It is well established, on the basis of experimental data, that the beta cells are concerned in the elaboration of insulin. Furthermore, there is evidence that the beta granules are intimately related to insulin. In the work reported in this paper we endeavored to show the effects of prolonged intravenous administration of dextrose U S P (d-glucose) on this specific granulation of the beta cells.

REVIEW OF LITERATURE

While the exact factors that stimulate the beta cells to elaborate insulin are not fully known, there is evidence that the level of the blood sugar is important. Anderson and Long¹ found that no secretion of insulin could be detected by assay of the fluid medium after an isolated pancreas had been perfused with blood low in glucose. When such a perfusion was made with blood containing large amounts of glucose, the perfusate indicated a great increase in secretion of insulin.

Houssay² observed that repeated or persistent hyperglycemia is an important factor in injuring the islets. He postulated that the hyperglycemia stimulates the beta cells excessively by inducing hyperfunction and hypersecretion, if long continued, this overstimulation leads to functional exhaustion and finally to injury and atrophy of the cells. Administration of sugar or of extracts of the anterior lobe of the pituitary gland or the thyroid gland causes elevation of the blood sugar.

From the Department of Pathology, University of Minnesota.

These investigations were supported in part by a grant from the Office of Naval Research made to Dr E. T. Bell and in part by the Graduate School of the University of Minnesota.

1 Anderson, E., and Long, J. A. *Endocrinology* **40**: 92, 1947.

2 Houssay, B. A. *Rev. med. panam.* **1**: 255, 1945.

Lukens, Dohan and Wolcott³ expressed the belief that the hyperglycemia induced by anterior pituitary extract was the important factor in damaging the islet cells. They found that if the hyperglycemia caused by injections of anterior pituitary extract in cats was prevented by concomitant administration of phlorhizin or insulin, the diabetes mellitus and lesions of the islets usually resulting from the administration of the anterior pituitary extract did not develop.

In 1936 Jacobs and Colwell⁴ maintained normal dogs on 50 per cent dextrose for as long as one hundred and sixty-eight hours, giving 0.7 to 4.5 Gm of dextrose per kilogram of body weight intravenously per hour. Death ensued, and the chief abnormalities observed at autopsy were hemorrhages in the pancreas and the pituitary gland with some degenerative changes. However, they did not stain the islets with technics that would demonstrate the beta cell granules.

Woerner,⁵ with continuous intravenous administration of dextrose, maintained hyperglycemia in guinea pigs and found mitotic activity, decreased granulation and hyperplasia of the beta cells. With higher levels of blood sugar, "exhaustion" of these cells was seen, manifested by degranulation or degeneration. He used the Lane-Bensley staining procedure, which we believe is less satisfactory than the Gomori⁶ technic.

Gomori, Friedman and Caldwell⁷ reported varying degrees of beta cell degranulation when transitory elevation of blood sugar was induced by a single intraperitoneal dose of dextrose, as normoglycemia returned, beta granulation was again observed. They offered the opinion that these histologic changes suggested functional activity of the beta cells, possibly related to the secretion of insulin as a response to the elevation of blood sugar.

Recently, Dohan and Lukens⁸ reported that diabetes mellitus had apparently been induced in cats with repeated intraperitoneal injections of dextrose, the pancreases showed hydropic changes in the beta cells. However no mention of the beta granules was made.

METHODS

Normal dogs were used. After the animal had been anesthetized with pentobarbital sodium, a plastic (polyethylene) tube, 3 mm in diameter, was inserted through the saphenous vein at the ankle and threaded up into the inferior vena cava.

3 Lukens, F. D. W., Dohan, F. C., and Wolcott, M. W. *Endocrinology* **32** 475, 1943.

4 Jacobs, H. R., and Colwell, A. R. *Am J Physiol* **116** 194, 1936.

5 Woerner, C. A. *Anat Rec* **71** 33, 1938, **75** 91, 1939.

6 Gomori, G. *Am J Path* **17** 395, 1941.

7 Gomori, G., Friedman, N. B., and Caldwell, D. W. *Proc Soc Exper Biol & Med* **41** 567, 1939.

8 Dohan, F. C. and Lukens, F. D. W. *Science* **105** 183, 1947.

This plastic tube was connected to the rubber tube from the bottle of dextrose solution by means of a three way stopcock and a 15 gage needle. To immobilize this lower extremity, the sciatic and femoral nerves to this leg were sectioned, and the entire leg was splinted and wrapped securely with ace bandages and adhesive tape. This permitted the animal to stand and move about freely in the cage. To protect the rubber tube that led from the bottle of fluid to the dog's leg, this tube was passed through a sturdy, thick pressure hose that was securely incorporated in the leg wrappings at one end and fixed to the cage at the other end. Thus, kinks and strains on the more delicate tube that carried the fluid were obviated. The dextrose solutions were infused continuously, and the dogs were permitted to eat and drink ad libitum.

Vitamin B complex,⁹ ascorbic acid (500 Gm) and menadione sodium bisulfite U S P¹⁰ (48 mg) were added daily to the intravenous fluids. Some 10 per cent dextrose in distilled water was given to the first dog, but because this concentration caused severe dehydration, the dogs that followed were given only 5 per cent dextrose in distilled water. Five per cent dextrose in sodium chloride solution was used occasionally in order to maintain fluid and electrolyte balance. Experiments were carried out successfully on 6 dogs, the seventh dog died twelve hours after the intravenous fluids were started and has not been considered in the data included in this paper. In each experiment, blood samples were drawn on a number of days for determination of sugar, carbon dioxide-combining power, fractional plasma proteins, urea nitrogen and chloride. The urine and the excreted sugar were not always measured because of technical difficulties. Each autopsy was made within one hour after death except as noted in the following pages. All tissues were fixed in Zenker's solution for hematoxylin and eosin stains. In addition, the pancreas was put in Bouin's fixative for Gomori's⁶ staining, and the liver was placed in absolute alcohol for Best's carmine stain for glycogen.

RESULTS

Duration of Experiments and Amounts of Dextrose Given—It was possible, by the technic employed, to maintain continuous intravenous administration of dextrose for four days in 2 dogs (1 and 5), seven days in 1 dog (4), eight days in 1 dog (6) and nine days in 2 dogs (2 and 3). The amount of dextrose and the rate of injection for each dog are given in table 1. The amount of dextrose injected per day varied from a minimum of 50 Gm (dog 5) to a maximum of 700 Gm (dog 1). The total amount of dextrose given each dog varied from 425 Gm (dog 5) to 2,250 Gm (dog 3).

Blood Sugar Levels (table 2)—When the blood sugar was determined within an hour after starting injection of dextrose, the levels were high, viz., 607 mg per hundred cubic centimeters in dog 1, 300 mg in dog 3 and 306 mg in dog 6. These initial high levels soon fell. This corresponds to the response usually obtained with the intravenous dextrose tolerance test, in which transitory initial rise is followed by normoglycemia in normal subjects. However, despite the fact that the animals were receiving large amounts of dextrose continuously, the value of the blood sugar remained within normal limits for three to seven days,

9 The preparation used was betasynplex (Winthrop) containing 20 mg of thiamine, 10 mg of riboflavin, 10 mg of pyridoxine hydrochloride, 10 mg of calcium pantothenate and 50 mg of nicotinamide.

10 The preparation used was hykinone® (Abbott), which contains "not less than 49 per cent menadione (C₁₁H₈O₂)"

after which hyperglycemia developed again and persisted until the experiment terminated. During this maximum sustained hyperglycemia the value of blood sugar ranged from 154 to 1,000 mg per hundred cubic centimeters.

TABLE 1—Amount and Rate of Administration of Dextrose

Dog	Sex	Weight, kg	Day	Volume of Dextrose Solution per Day, Liters	Concen- tration of Dextrose Solution, per Cent	Gm Dextrose per Day	Gm Dex- trose per kg per Day
1	F	17.7	1	5.0	5	250	14.1
			2	11.0	5	550	31.0
			3	6.0	5	300	16.9
			4	4.0	10	400	22.6
			5	4.0	10	400	22.6
			Total	30.0		1,900	
2	M	12.0	1	2.0	5	100	8.3
			2	2.0	5	100	8.3
			3	3.0	5	150	12.5
			4	5.0	5	250	20.8
			5	7.0	5	350	29.1
			6	4.5	5	225	18.7
			7	4.0	5	200	16.6
			8	4.0	5	200	16.6
			9	2.0	5	100	8.3
			Total	33.5		1,675	
3	M	16.8	1	3.0	5	150	8.8
			2	3.0	5	150	8.8
			3	4.0	5	200	11.9
			4	4.5	5	225	13.4
			5	6.0	5	300	17.8
			6	11.0	5	550	32.7
			7	6.5	5	325	19.3
			8	4.0	5	200	11.9
			9	3.0	5	150	8.8
			Total	45.0		2,250	
4	F	13.6	1	2.0	5	100	7.35
			2	4.0	5	200	14.7
			3	4.0	5	200	14.7
			4	7.0	5	350	25.7
			5	5.7	5	285	20.9
			6	4.0	5	200	14.7
			7	5.0	5	250	18.3
			Total	31.7		1,585	
5	F	11.1	1	1.5	5	75	6.75
			2	3.0	5	150	13.5
			3	2.0	5	100	9.0
			4	2.0	5	100	9.0
			Total	8.5		425	
6	F	11.3	1	2.5	5	125	11.1
			2	4.0	5	200	17.7
			3	4.0	5	200	17.7
			4	4.0	5	200	17.7
			5	1.0	5	50	4.4
			6	5.0	5	250	22.1
			7	3.0	5	150	13.3
			8	6.0 cc 400 cc 600 cc	50 20 20	20 50 120	22.1 10.6
			Total	24.5		1,395	

Blood Urea Nitrogen, Carbon Dioxide-Combining Power, Chlorides and Plasma Proteins—Except for dog 1, in which there was a marked decrease in the carbon dioxide-combining power (28 volumes per cent on the day before death) and dog 6, in which the plasma chlorides were reduced to 422 mg per hundred cubic centimeters on the last day of the experiment, there were no noteworthy alterations.

in blood urea nitrogen, carbon dioxide-combining power, chlorides or plasma proteins. These are shown in table 2.

Cause of Death—In each experiment, the dogs fared well for the first two days, but after this, increasing lassitude and anorexia appeared. The condition of each dog was then one of gradual decline, but the exact cause of death was not apparent in all cases. In dogs 2 and 4 there was marked pulmonary edema, indicating overhydration.

Autopsy Observations—Macroscopic Changes (table 3). In each instance autopsy was performed as soon after death as was practical, in most cases it was performed within three to four hours. The macroscopic findings in each

TABLE 2—Results of Chemical Examination of Blood

Dog	Day	Blood Sugar, Mg per 100 Cc	Blood Urea Nitrogen, Mg per 100 Cc	CO ₂ - Combining Power, Volume per Cent	Chlorides, M _{Eq} per 100 Cc	Proteins, Gm per 100 Cc		
						Albumin	Globulin	Total
1	1	607*	10	55				5.2
	2	85	2		653			
	3	159	10	28	622			5.5
	4	{ 1,000 762						
2	2	100		47	617			...
	4	114	3	44	673	3.1	2.1	5.2
	5	135						
	7	224	7	40	600	2.7	1.5	4.2
3	8	254						
	1	300*	10	44	620			6.4
	4	140	5		667	3.0	1.9	4.9
	6	236	6	49	494	2.9	2.0	4.9
4	7	130†						
	9	203						
	2	102	4	39	654	3.5	2.3	5.8
	4	67	13	55	550			5.6
5	7	176	7	59	556	3.5	1.8	5.3
	2	83	8	44	670	3.9	1.7	5.6
	3	154‡						
6	1	306*			612			7.6
	2	93						
	3	113	3	43	582	3.4	2.5	5.9
	4	125						
	6	106	5	41	494	3.6	2.1	5.7
	7	71	5	49	562	3.9	2.6	6.5
	8	373	5	48	422	2.7	2.0	5.7

* Blood was taken within one hour after start of intravenous injection of dextrose.

† Blood was taken one hour after intravenous injection of dextrose had stopped.

‡ Blood was taken during a convulsion.

of the dogs were not marked or uniform. In dog 1 the pancreas was congested, the kidneys showed cloudy swelling, the lungs were slightly congested and there were small hemorrhages in the spleen. In dog 2 there were ascites, hydrothorax and a rather marked hemorrhagic edema of the lungs. The pancreas appeared normal. In dog 3 slight congestion of the pancreas and slight swelling of the kidneys were observed, with a few hemorrhages in the lungs and beneath the serosa of the intestines. In dog 4 there were marked edema and small hemorrhages of the lungs and multiple petechiae of the colon, but the pancreas appeared normal. In dog 5, apart from an abscess and cellulitis at the site of the section of the femoral nerve, the findings were not noteworthy. Dog 6 was examined about five hours after death and the essential findings were dehydration of all tissues, maceration of the skin and muscles of the leg in which the intravenous tube had been introduced and central congestion of the liver.

TABLE 3—*Observations**

Dog	Macroscopic	Microscopic
1	Autopsy within one half hour after death Pancreas—congested Liver—friable, pale tan color Kidneys—cloudy, pale Lungs—slight congestion Spleen—small hemorrhages Large intestine—pasty red contents	Pancreas—Hematoxylin and eosin stain only occasional islets intact, most show hemorrhagic necrosis, congestion, edema, and hemorrhage in lobules and interstitial tissue Gomori stain complete degranulation of beta cells, most islets necrotic Liver—Hematoxylin and eosin stain all liver cells completely clear and hydropic no visible necrosis Glycogen stain large amounts of glycogen shown Lung—congestion of septums, slight edema in alveoli, occasional bronchi filled with polymorphonuclear leukocytes Spleen—congestion and small areas of hemorrhage Kidney—congestion, small petechiae
2	Autopsy at 3 to 4 hours after death Body swollen and crepitant Lungs—hemorrhagic edema Ascites and plural effusion Pancreas—no changes	Pancreas—Hematoxylin and eosin stain a few petechiae seen in the islets and acinous tissue, early postmortem changes Gomori stain complete degranulation of beta cells of islets Liver—Hematoxylin and eosin stain no significant abnormality, early postmortem changes Glycogen stain no glycogen seen Kidney—mild interstitial edema, mild post mortem changes
3	Autopsy within 1 hour after death Pancreas—slightly congested Lungs—few hemorrhages, no edema Kidneys—slightly swollen Scattered serosal petechiae	Pancreas—Hematoxylin and eosin stain a few petechiae in islets and in acinous and interstitial tissue, congestion Gomori stain complete degranulation of beta cells in islets Liver—Hematoxylin and eosin stain most lobules contain largely hydropic cells, others are normal, slight central congestion in lobules Glycogen stain large amounts of glycogen shown Kidney—slight interstitial edema, otherwise normal Lung—subpleural petechiae in alveoli, otherwise normal Heart—normal except for occasional petechiae
4	Autopsy within 1 or 2 hours after death Pancreas—negative Lungs—marked edema and small hemorrhages Colon—petechiae and bloody contents	Pancreas—Hematoxylin and eosin stain no abnormality seen Gomori stain complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain a few lobules with some hydropic liver cells, others are normal Glycogen stain moderate amount of glycogen seen at periphery of lobules Kidney—slight congestion and a few petechiae Lung—congestion of septums and edema in alveoli a few petechiae seen
5	Killed by blow to head and autopsy performed at once Abscess and cellulitis at site of femoral nerve section No other gross changes	Pancreas—Hematoxylin and eosin stain no abnormality seen Gomori stain complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain no abnormality seen Glycogen stain moderate amount of glycogen seen Lung—congestion of septums, slight atelectasis in some areas
6	Autopsy at not over 5 hours after death All tissues appear dehydrated Liver—nutmeg appearance Maceration of leg with intravenous tubes inserted No other gross changes	Pancreas—Hematoxylin and eosin stain slight congestion in the islets otherwise no abnormalities Gomori stain complete degranulation of all beta cells of islets Liver—Hematoxylin and eosin stain mild central congestion of lobules Glycogen stain moderate amounts of glycogen seen at periphery of lobules only Lung—congestion of septums and edema in alveoli dense exudate of polymorphonuclear leukocytes in bronchi

* Only the significant observations are tabulated

Microscopic Changes (table 3) The routine hematoxylin and eosin stains of the pancreas showed no significant abnormalities of the acinous or islet tissues except in dog 1. In this animal, there were numerous diffuse hemorrhages throughout the parenchyma, and nearly every islet showed hemorrhagic necrosis. The islet cells had disappeared, and the area was replaced by hemorrhage. Only an occasional normal islet was seen. In the other dogs, only minimal changes in the islets were seen in hematoxylin-eosin preparations. With the Gomori stain, however, a complete degranulation of all the beta cells was found in every islet of each animal.

The lungs showed only edema and congestion. Occasionally there were erythrocytes in the alveoli, and a few bronchi showed accumulations of polymorphonuclear leukocytes.

The most marked changes found in the liver were those in dog 1, in which all the parenchymal cells were completely clear and hydropic, but there was no visible necrosis. Many of the cells appeared swollen, and except for the cell outline, no cell substance could be seen. These changes were found to a lesser extent in dog 3, in which a majority of the lobules showed largely hydropic changes. The livers of the other dogs were essentially normal except for varying amounts of minimal postmortem change. As determined by Best's carmine stain, glycogen was found in large amounts in the liver of dog 1 and in lesser amounts in the livers of the other animals.

No significant alterations were observed in the kidneys, the spleen or other organs.

COMMENT

It appears that prolonged hyperglycemia stimulates the beta cells excessively and that the overstrain exhausts the supply of insulin and causes degranulation. The only example of necrosis of the islet cells was seen in dog 1. The necrosis might have been due to overstimulation, but other explanations are possible. It is interesting that except for the initial transitory hyperglycemia within a few hours after the infusion of dextrose was begun, normoglycemia was maintained for three to seven days before persistent hyperglycemia developed. This might be regarded as a period in which the pancreas and other organs involved in carbohydrate metabolism could still meet the great demands placed on them, however, when this "overwork" was continued, islet "exhaustion" ultimately developed, and the blood sugar again became elevated. This might be regarded as an eventual decompensation of the functional ability of the islets to produce sufficient insulin, following the latent period in which the pancreas could compensate for the massive doses of dextrose and still maintain the normoglycemic state. Before such decompensation is manifested by hyperglycemia, degranulation occurs—apparently as an indication of pancreatic hyperfunction.

The cause of the hyperglycemia of diabetes mellitus is unknown, but the results of these experiments indicate that persistent hyperglycemia may in turn cause definite damage to the beta cells of the islets of Langerhans. This may be of significance in the management of patients with diabetes mellitus, in whom a prolonged state of

relatively poor control of the blood sugar may add to the damage of the beta cells which was present when the diabetes first became manifest

SUMMARY

Dogs were given large amounts of dextrose, varying from 425 to 2,250 Gm, continuously by intravenous injection for as long as nine days

After an initial transitory rise in blood sugar there was a latent period of normoglycemia for three to seven days until sustained hyperglycemia developed

In all instances the beta cells of the islets of Langerhans were degranulated. In dog 1, given massive amounts of dextrose to a total of 1,900 Gm in four days, there was, in addition, hemorrhagic necrosis of the islets

It is postulated that these changes are an indication of functional overstrain or "exhaustion" of the beta cells. This leads to the premise that the beta granules are related to the production of insulin

The results of these experiments indicate that in clinical diabetes mellitus poor control of the blood sugar with resultant hyperglycemia may result in further damage to the beta cells and consequent increased severity of the diabetic state

HISTOCHEMICAL DEMONSTRATION OF A LIPASE IN CARCINOMA OF THE LUNG

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OF A LARGE series of cancers, including both carcinoma and sarcoma, studied for lipase activity by Gomori, only 2, which were diagnosed as carcinoma of the esophagus and hepatoma, respectively, had this enzyme in their cells. A few unidentified stroma cells of an atypical seminoma were also shown to contain lipase¹. No statement was made by Gomori as to the origin of the tumors which showed no lipase within their cells.

We have recently studied 6 cases of bronchogenic carcinoma in which wide variation of the lipase content of the tumors was found, which we report in the hope that our findings in this relatively unexplored field will be of use to students of this subject.

MATERIAL AND TECHNIC

With the exception of 1 case in which tissue was removed two hours after death at autopsy, the lungs which we studied were dissected within ten minutes after they were removed from the patient. Lipase was demonstrated in paraffin sections of acetone-fixed tissues by the Gomori technic¹. The water-soluble ester of palmitic acid² was used, and the pH of the substrate was adjusted to 7.3 to 7.4 with 4 per cent sodium hydroxide. The sites of lipase activity were stained by transforming the calcium palmitate formed into the corresponding lead salt and blackening with hydrogen sulfide. Control sections that received identical treatment except that they were not incubated in the substrate were routinely made. This was done in order to rule out calcium salts, melanin, lipochrome, hemosiderin and other pigments which could be confused with the lipase.

The remaining lung and tumor tissue were fixed in 4 per cent formaldehyde solution and stained with hematoxylin and eosin.

OBSERVATIONS

In areas of the lungs relatively uninvolved by disease the sites of lipase activity were similar to those found by Gomori¹. The bronchial epithelium showed lipase activity in a patchy fashion. The epithelium of the ducts to the bronchial glands

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1 Gomori, G. Arch Path **41** 121, 1946

2 Tween 40 was used, obtained from the Atlas Powder Company, Wilmington, Del.

was usually strongly positive for lipase, and the cells of the bronchial glands showed both positive and negative staining cells. The attached septal cells of the alveolar walls were negative for lipase, desquamated epithelial cells were usually, but not always, positive. Muscle, cartilage and vascular and lymphatic tissue were negative. Where areas of fibroblastic connective tissue were seen, in several lungs faint diffuse staining was present.

The carcinomas observed were all of the epidermoid type and varied morphologically in minor degrees among themselves and in different areas of the same tumors. The following descriptions give the pertinent observations for each case.

CASE 1—J. L. B., aged 41, a white truck driver, had respiratory symptoms for six months. He died from a primary tumor which arose from the bronchus of the upper lobe of the right lung and from widespread metastasis to the brain, the liver, the adrenal glands, the kidneys, bones, the thyroid gland and lymph nodes. The autopsy was performed two hours after death. Sections showed the carcinoma in the lung and the liver to be composed of masses of undifferentiated moderate-sized cells with rather regular nuclei. The nucleoli were not prominent. Mitotic figures were infrequent.

No lipase activity was demonstrated in the carcinoma of the lung and the liver. Lipase activity was present in the macrophages of the lung adjacent to the carcinoma and in the hepatic cells of the liver adjacent to the carcinoma (fig. 1). The slides had been incubated in the substrate for eighteen hours.

CASE 2—J. G. G., aged 60, a white coal miner, had respiratory symptoms for two months. A sessile, fungating epidermoid carcinoma, grade 2, was found arising from a bronchus leading to the mesial inferior aspect of the lower lobe of the left lung. The tumor occluded the bronchus at one point. Sections showed the transition from normal bronchial epithelium to well differentiated cancer cells with good polarity. The outer cells of the invading tumor were columnar, while the cells in the interior of the tumor had squamous characteristics. In some areas the interior of the invading masses of tumor formed cystic areas filled with cellular debris. In other areas of the tumor jumbled masses of smaller cells with less cytoplasm invaded the bronchial wall. Mitotic figures were frequent in both varieties of cells. No metastases were found in the hilar lymph nodes.

The smaller cells of the tumor, the bronchial epithelial cells and the ducts to the bronchial glands showed lipase activity. The more differentiated invading cells revealed no lipase activity (figs. 2 and 3). The slides had been incubated in the substrate for forty hours.

CASE 3—C. S., aged 29, a white housewife, had respiratory symptoms for seventeen months. A sessile epidermoid carcinoma, grade 2, measuring 0.75 cm., in its greatest dimensions, was found arising from the bronchus of the upper lobe of the right lung. Sections showed small cells with dark angular nuclei, arranged in groups and strands. Mitotic figures were infrequent. The tumor had invaded the muscle of the wall of the bronchus but had not invaded an adjacent lymph node. No metastases were noted in the hilar lymph nodes.

No lipase activity was demonstrated in the neoplastic tissue, although adjacent bronchial glands and bronchial epithelium showed sites of lipase activity. The sections had been incubated in the substrate for eighteen hours.

CASE 4—I. C. W., aged 58, a white locomotive fireman, had a history of asthma and asthmatic bronchitis for many years. Two years prior to his pneumonectomy, an unexplained shadow was found by roentgenogram in the apex of the lower lobe of the right lung. A friable polypoid epidermoid carcinoma, grade 3, arising at the origin of the bronchus to the posterior lateral portion of the middle lobe of

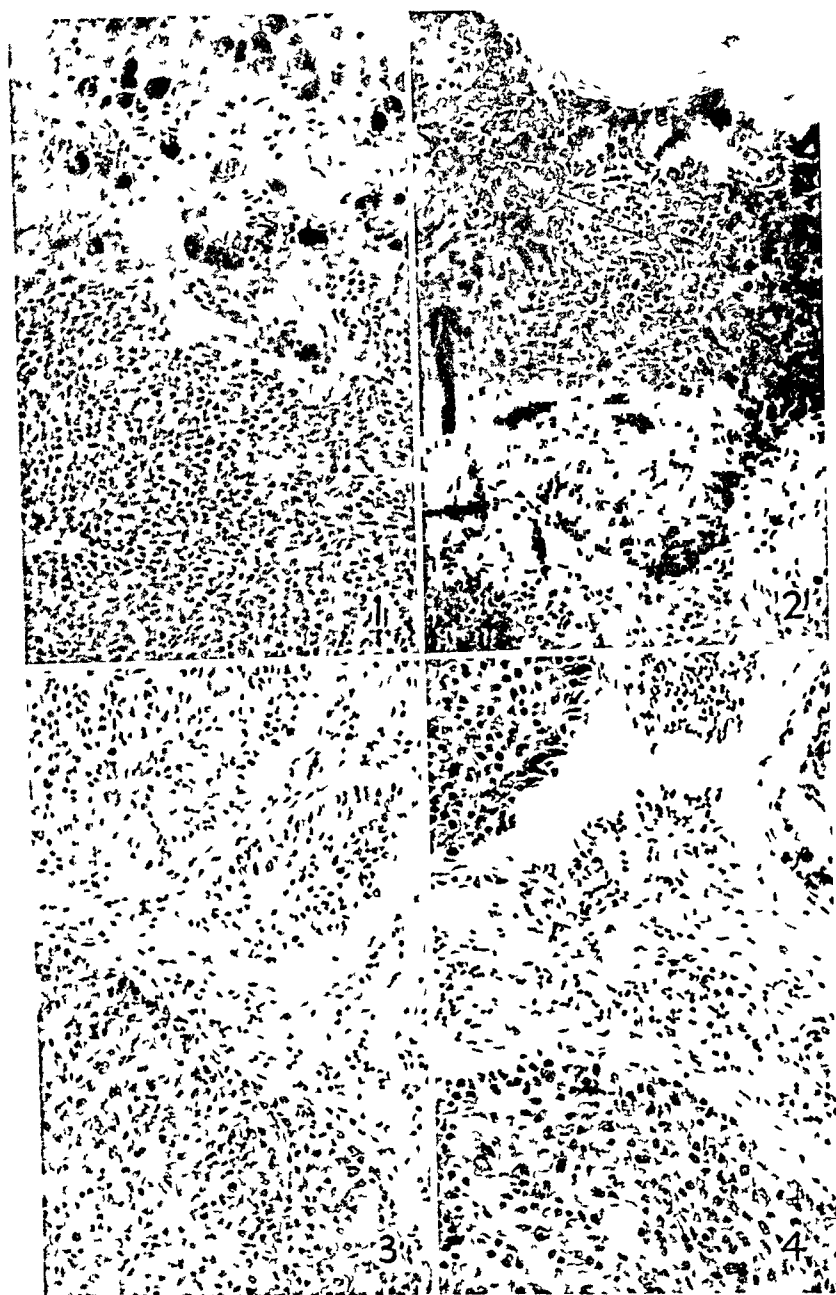


Fig 1 (case 1)—A rapidly growing carcinoma in the lung that shows no lipase activity. The macrophages in the adjacent tissues show abundant lipase activity and appear black in the photograph. The nuclei of the cells are counter-stained with hematoxylin.

Fig 2 (case 2)—Both the carcinoma and the adjacent bronchial epithelium show lipase activity.

Fig 3 (case 2)—This is the same carcinoma as the one seen in figure 2. The block of tissue was taken from the deeper portions of the tumor. Some portions of the carcinoma show sites of lipase activity while other areas of the carcinoma are without lipase activity.

Fig 4 (case 4)—The carcinoma shows lipase activity. The lymphocytes appear dark in the photograph because they have been stained with hematoxylin.

the right lung was found. The polypoid growth obstructed the orifice of a small neighboring bronchus. The hilar lymph nodes showed extensive metastasis. Sections showed areas of spindle-shaped cells having prominent intercellular processes. Other areas showed cells of a large squamous variety with large, irregular nuclei.

In both varieties nucleoli were not prominent. Some of the invading masses of cells had a tendency toward a columnar cell arrangement; the outermost layer of cells, with inner cystlike areas filled with cellular debris. Other areas of the tumor showed cells with a complete loss of polarity. Mitotic figures were relatively infrequent. The metastases in the lymph nodes were composed of squamous epithelial cells.

There were sites of lipase activity in the neoplastic cells in all sections studied (fig. 4). The bronchial epithelium and the bronchial glands failed to show lipase activity. An occasional alveolar macrophage was positive. The sections had been incubated in the substrate for twenty-three hours.

CASE 5—W. W. R., aged 68, a white farmer, had respiratory symptoms for six months. An epidermoid carcinoma, grade 2, arose from the wall of the bronchus to the lower lobe of the right lung, produced stenosis of a bronchus and was 10 cm in diameter in its greatest dimensions. Microscopically, the tumor was characterized by well defined groups of squamous cells having moderate-sized and giant nuclei with multiple large nucleoli. Metastases were found in the hilar lymph nodes.

No lipase activity was demonstrated in the tumor cells, although the bronchial epithelium and the adjacent macrophages were strongly positive. The time of incubation of the sections was twenty-two hours.

CASE 6—W. J., aged 55, a white coal miner, had respiratory symptoms for one year. A large, fungating, friable epidermoid carcinoma, grade 2, was found arising from the left bronchus. The tumor extended 6 cm into the pulmonary tissue and obstructed several bronchi. No metastases were found in the hilar lymph nodes. Sections showed squamous epithelial cells with large prominent nucleoli invading the bronchial wall. A few areas were composed of smaller basophilic cells with much less cytoplasm. Mitotic figures were not frequent.

Lipase activity was demonstrated in the superficial squamous cells of the tumor. Other areas of squamous cells and the smaller basophilic cells of the tumor showed no lipase activity. Macrophages and the epithelium of the ducts of the bronchial glands showed lipase activity. The sections had been incubated in the substrate for twenty-two hours.

COMMENT

Gomori, in histochemical studies of lipase activity, demonstrated lipase within the cells of only 2 cancers of a large series. These 2 tumors arose from the mucosa of the esophagus and the liver cells, respectively. It is of interest that the normal cells of these tissues regularly contain this enzyme. Lipase activity has so far been demonstrated within tumor cells only in tumors arising from tissues which normally contain lipase.

SUMMARY

Using Gomori's technic, we studied 6 cases of bronchogenic carcinoma and found abundant evidence of lipase activity in 3 of them. No correlation of lipase activity and morphologic or biologic character of the tumor was possible.

HEALED DISSECTING ANEURYSM

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PHILADELPHIA

SINCE Shennan¹ reported on 300 cases of dissecting aneurysm of the aorta, the literature on this topic has become rather voluminous. A report of an isolated case, therefore, would not seem justified unless it exemplified some unusual feature such as that encountered in this case, which is reported because of the nature of the anomaly and the problems posed in its analysis.

J E, a 58 year old Jamaican Negro, residing in Panama for thirty-six years, was brought to Gorgas Hospital on June 6, 1944, complaining of a sudden onset of epigastric pain and vomiting. He stated that he had previously been entirely well, never having undergone any illness, operation or injury. His parents and nine siblings were living and well.

On admission, the blood pressure was 140 systolic and 80 diastolic, with "normal" pulse and respiration rates and temperature. He did not appear in distress and was mentally clear. Heart, lungs and abdomen seemed normal. On admission laboratory examination revealed a hemoglobin content of 70 per cent, with 3,920,000 red cells. The white cell count was 13,900, 83 per cent of which were neutrophilic granulocytes. An electrocardiogram taken the following day revealed low T waves and slurred QRS complexes, but the tracing was not considered diagnostic. Roentgen examination of the chest and abdomen, intravenous urography and a barium sulfate enema disclosed no abnormality. Urinalysis revealed no abnormal findings.

For the first hospital week he complained of a steady periumbilical pain, which was occasionally relieved by antispasmodic drugs. A 2:1 heart block was noted, but otherwise subsequent electrocardiograms were unchanged. The blood pressure remained at 160 systolic and 80 diastolic. The impression at this time was "probably myocardial infarction." After two weeks he became symptom free and was discharged on July 31. At that time the studies were inconclusive, but discharge was effected because of available air transportation to the United States Army base where he was employed.

He was again admitted twenty months later, Feb 21, 1946. At that time he complained that there had been mild nocturia for six months and severe headache for four days. The headache was followed by severe pain on the right side of the body, impaired vision and weakness, more noticeable in the left leg.

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¹ Shennan, T. Dissecting Aneurysms, Medical Research Council, Special Report Series, no 193, London His Majesty's Stationery Office, 1934.

At this time the blood pressure was 240 systolic and 150 diastolic, and the heart was enlarged to percussion. The rhythm was regular, and no murmurs were noted. The lung fields appeared normal, and the abdominal and neurologic examinations gave negative results.

The hemoglobin was 64 per cent and the red cell count was 3,740,000. The white cell count was 7,600, with 65 per cent neutrophils. The urine had a specific gravity of 1.016 and showed albumin (4 plus), with hyaline casts and red and white blood cells. The Wassermann test was negative. The blood nonprotein nitrogen was reported as 48.1 mg and the creatinine 3.4 mg per hundred cubic centimeters. Two days later the nonprotein nitrogen was 64.2 mg. A fundoscopic examination was reported as showing a "grade III hypertensive retinopathy."

The blood pressure remained elevated, and the patient became progressively disoriented and comatose. March 1, 1946, the temperature rose to 106.2 F, and the blood pressure fell to 64 systolic and 50 diastolic. He died the same day. The clinical impression was "hypertensive cardiovascular disease with encephalopathy."

Autopsy (twelve hours after death)—Additional laboratory work on post-mortem material revealed specific gravity of the urine 1.017, with albumin (2 plus) and a few red cells and granular casts, blood nonprotein nitrogen 154.8 mg, urea nitrogen 120.0 mg, creatinine 8.2 mg and glucose 137.0 mg per hundred cubic centimeters. Wassermann tests of blood and spinal fluid were negative.

The external and general internal examinations disclosed essentially noncontributory conditions. The heart weighed 430 Gm, with a left ventricular wall measuring 18.0 mm in thickness. The coronary arteries showed moderate patchy atherosclerosis. The aorta, being the point of interest, will be described in detail later. The lungs showed moderate edema, congestion and patchy areas of bronchopneumonia. The liver was slightly enlarged, weighing 1,750 Gm, and was moderately congested. The gallbladder and the biliary tract were not remarkable. The spleen appeared slightly fibrotic. The kidneys were slightly small, each weighing 140 Gm and showed evidence of arterial and arteriolar sclerosis. There was moderate polyposis of the sigmoid colon. The remainder of the gastrointestinal tract, the pancreas, the adrenal glands and the genital organs were not remarkable. The brain weighed 1,350 Gm and showed slight edema plus two small areas of ischemic necrosis, one in each lenticular nucleus. There was moderate cerebral arteriosclerosis.

The aorta showed slight atherosclerosis of the ascending portion. In the descending portion, 2.0 cm distal to the ostium of the left subclavian artery there was an anomalous orifice, measuring 3.0 by 1.4 cm, and located on the left posterolateral aspect. This orifice communicated with an anomalous channel which traversed the length of the aorta along its left posterolateral aspect. Immediately distal to its origin, the anomalous channel showed a saccular aneurysmal dilatation, measuring 5.0 by 4.5 cm. This was traversed by pearly white delicate cords. A number of similar cords were noted along the course of this channel, running transversely and located at the inferior angle formed by the septum between the two vessels.

Arising from this accessory channel, in its thoracic portion, were a number of intercostal arteries, each completely patent. Similarly the left renal artery was seen to have its origin from the false channel, being at the same level as the normally located right renal artery. The terminus of this accessory aortic channel was the left common iliac artery, the origin of which could be considered similar to the origin of the left renal artery. At the point corresponding to the bifurcation of the aorta there was a communication between the two aortic channels which was covered by a delicate valvelike membrane that extended down into the left



Fig 1—Anterior view The normal aortic channel Note the hypertrophy of the left ventricle and the similar, relatively normal appearance of the kidneys Probes are inserted into the ostium of the anomalous channel and the communication leading to the left common iliac artery ($\times 05$)

common iliac artery for a distance of 12 cm and attached on its medial wall. Thus, the circulation of the left common iliac artery was derived in the main from the anomalous channel, although some blood could come from the normal channel, since the valve was not completely obstructive.

Atheromatous plaques, some with superficial ulceration, were seen on the surfaces of both "aortas." The majority of these were in the anomalous channel, where they occurred in groups, particularly in the area near the renal artery. The two renal arteries, likewise the common iliac arteries, were of normal caliber, and each was similar in appearance to the other.

In general appearance the two aortic channels were of the nature of a double lumen tube, divided by a septum. Aside from the branches indicated, the major vessels arose in a normal fashion.

The kidneys were similar to each other in the changes and in the degree of change. The findings were those usually associated with hypertensive disease. There was moderate arteriosclerosis and a rather marked, though focal, hyaline arteriolosclerosis. Necrotizing arteriolar changes were not noted. In the areas of vascular involvement, the glomerular tufts showed changes varying from ischemia and collapse to fibrosis and hyalinization. Nearby, the convoluted tubules were enlarged and dilated. Foci of lymphocytes were present in the stroma.

The sections of the junction of the left renal artery and the anomalous aortic channel showed a direct continuity of all layers. There was moderate atherosclerotic deposit, with slight peripheral hemorrhage. The innermost medial fibers appeared slightly small and coursed irregularly, and there was moderate fibrosis. The internal elastic lamina was seen only in the renal artery portion. It appeared to end blindly at the ostium. There was lymphocytic streaking in the adventitia.

Histologic studies were made of the aorta, the anomalous channel and the septum between, using hematoxylin-eosin, Van Gieson and elastic tissue stains. These revealed similar components in each vessel, namely, intima, media and adventitia. The septum was composed of medial fibers, bordered on each side by intima. Atheromatous changes were prominent in both vessels. The atheromas of the anomalous channel were bordered by small hemorrhages. There was no evidence of cystic medial necrosis. The medial fibers forming the internal half of the wall and septum of the anomalous channel were small and irregularly formed. In both vessels there were linear collections of lymphocytes in the media and perivascular collections in the adventitia.

On completion of the autopsy, the immediate impression was that of healed dissecting aneurysm. However, rapid acknowledgment of disturbing features was made. It was thought probable that a dissecting aneurysm involving the renal artery would so impair the renal circulation that gross manifestations would result. In this case the two kidneys were remarkably similar in size and appearance, without evidence of infarctions. Thus, the situation of having a normal renal artery arising from an anomalous aortic channel and supplying an unremarkable kidney suggested the possibility that the original impression was incorrect. It seemed, at that time, improbable that an aortic dissection could occur across, rather than along, a vessel such as the renal, so as to reimplant this artery in the new channel without embarrassing the circulation.

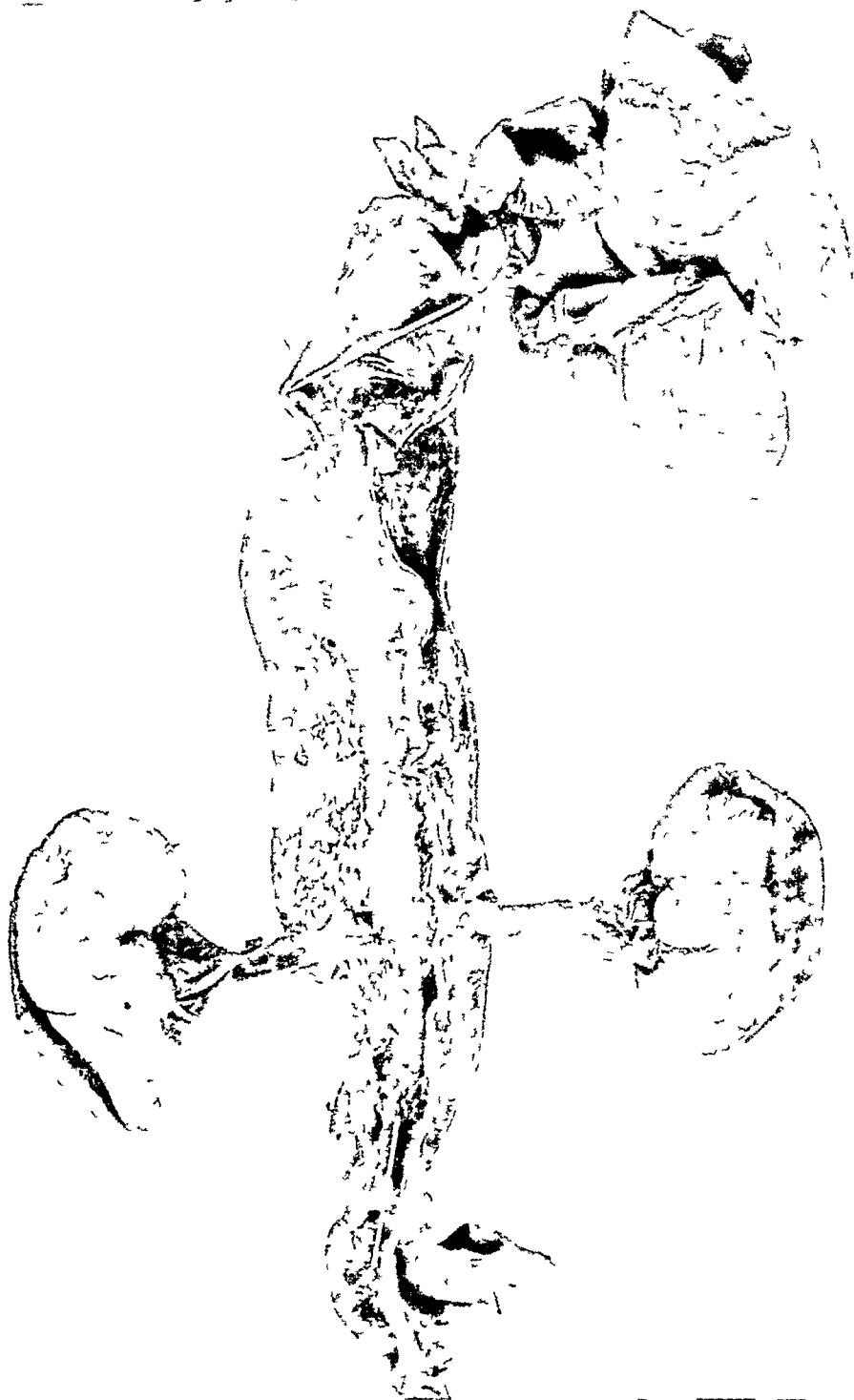


Fig 2—Posterior view The anomalous aortic channel Note (and compare with figure 1) the origin and the caliber of the left renal artery and the degree of atherosclerosis Probes are inserted into the saccular dilatation and beneath the membrane covering the ostium of the left common iliac artery ($\times 0.5$)

A review of the standard textbooks and the available literature was noninformative. A consultation among members of the laboratory and hospital staffs resulted in the opinion that we were dealing with a congenital anomaly, a double aorta of a lower phylogenetic form resulting from failure of fusion of the primitive paired aortas. Several authorities, consulted by letter, concurred in this opinion.

With the apparent presence of an extremely rare anomaly, publication was considered at that time but was deferred because there still remained a certain element of doubt as to the tenability of the diagnosis, particularly since many early pathologists misinterpreted healed dissecting aneurysms as congenital lesions.

Almost at the same time as this case was under observation, Cassidy and Pinniger² were reporting a case of healed dissecting aneurysm in which the right renal and testicular arteries arose from the false channel. No mention was made of the caliber of the renal artery or of a comparison of the vessels on the two sides. However, the right kidney did show several gross infarcts, which would indicate some impairment of the circulation. This report recently became available and immediately reopened the discussion.

Once again the literature was combed for similar or pertinent reports and correspondence was undertaken with numerous authorities. The literature surveyed was, as before, noncontributory, and the opinions ventured by the consultants were of an indefinite and inconclusive nature. The predominant opinion was that this did represent a healed dissecting aneurysm, despite the anomalous features. However, a congenital lesion was not considered as being entirely excluded. It was decided, therefore, to consider this as an unusual type of dissecting aneurysm. The causation was not clear. Some of the histologic features suggested a diagnosis of syphilis, despite negative serologic tests. An arteriosclerotic factor must be considered. There was no evidence of the cystic medial necrosis most commonly reported in conjunction with dissecting aneurysms.

It might be of interest to consider and compare the degrees of atheromatous degeneration in the two aortic channels. That present in the normal aorta was moderate in amount. However, as was noted, the plaques present in the anomalous channel were more numerous and showed a peculiar grouping. From the clinical standpoint the dissecting process was of at least twenty months' duration. Thus, it would appear that a greater degree of atheromatous deposit can occur in a newly formed vessel in a relatively short time than had developed during the total life of the patient in a normal vessel of similar caliber, but bearing a greater burden.

2 Cassidy, M, and Pinniger, L. L. *Brit Heart J* 8 130, 1946

It was shown that the last twenty months of life were accompanied clinically by rapidly increasing hypertension. Some correlation between hypertension and atheroma is a well known concept (Boyd³). It is conceivable that a greater degree of atheroma could form in a vessel wall which was not previously normal, if one is to consider the possibility of a congenital lesion, or in the walls of a vessel produced as a result of some destructive process such as a dissecting aneurysm.

The mechanism by which the renal artery was reimplanted without embarrassing the circulation, leaving an essentially normal vessel, is not clear. It is of interest, however, to consider to what extent the healing process can occur in the dissecting aneurysm.

SUMMARY

An unusual case, most probably one of a healed dissecting aneurysm of the aorta, is reported.

An indication of the problems encountered in the analysis of obscure lesions has also been briefly presented.

³ Boyd, W. A Textbook of Pathology, Philadelphia, Lea & Febiger, 1943, pp 394 and 397.

ISOALLERGIC ENCEPHALOMYELITIS PRODUCED IN GUINEA PIGS

Via Intramuscular and Intraperitoneal Injection of Antigen

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THE PROBLEM of the genesis of postinfectious encephalitis, which first arose from clinical observation of encephalomyelitis following antirabies treatment and postvaccinal encephalitis, prompted some of the initial investigations on the relationship of allergy to the nervous system. From a clinical standpoint several authors had already advanced the theory of an allergic origin of certain neurologic inflammatory diseases. Using various types of brain suspension, Rivers, Sprunt and Berry,¹ Rivers and Schwentker² and Ferraro and Jervis³ were able to produce in rabbits and monkeys an encephalomyelitis characterized by perivascular cellular infiltration and demyelination. Jervis, Ferraro and the Kopeloffs⁴ succeeded, on the other hand, in determining in the brain of the monkey, as a result of repeated sensitizations with egg white, the Arthus phenomenon at the site of the intracerebral injection of the antigen, and at a distance, in the same brain, a form of encephalomyelitis closely resembling histologically the one obtained by Rivers.

One of us, Ferraro,⁵ in 1944 described histologic changes occurring in the brain of a patient who had died of scarlet fever encephalitis and related these changes to an allergic brain reaction. In addition, on the basis of neuropathologic changes in human demyelinating diseases, which he compared with his experimental results, he emphasized the

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2 Rivers, T M, and Schwentker, F F J Exper Med 61 689, 1935

3 Ferraro, A, and Jervis, G Arch Neurol & Psychiat 43 195, 1940

4 Jervis, G A, Ferraro, A, Kopeloff, L, and Kopeloff, N Arch Neurol & Psychiat 45 733, 1941

5 Ferraro, A J Neuropath & Exper Neurol 3 239, 1944

relationship between demyelinating diseases and allergic reactions of the brain ⁶

With the Rivers brain suspension technic many months were required to determine experimental encephalomyelitis Freund and McDermott ⁷ devised a technic in which adjuvants were added to the antigen, a modification which shortened the period of incubation for the production of the inflammatory reaction in the brain and spinal cord

Freund's technic was first used in monkeys by Morgan ⁸ and, independently, by Kabat, Wolf and Bezer ⁹ We ¹⁰ succeeded in reproducing in monkeys chronic aspects of the encephalomyelitis by using small doses of the antigen Acute encephalomyelitis has been reproduced in rabbits by Morrison ¹¹ and in guinea pigs by Freund, Stern and Pisani, ¹² the Kopeloffs, ¹³ Alvort, ¹⁴ Jervis and Koprowsky ¹⁵ and ourselves ¹⁶

In 1946 the Kopeloffs ¹⁷ reported the presence of anti-brain antibodies in the serums of monkeys treated with many injections of alcoholic extract of sheep brain incorporated in water-in-oil emulsion, following Freund's technic

The original theory advocating specificity in the sense of organ selectivity rather than organ species preference in the production of the encephalomyelitis seemed to receive confirmation In other words, an emulsion of either homologous or heterologous brain is able to produce an inflammatory reaction in the nervous system of the host Anti-brain antibodies resulting from the injection of the brain emulsion plus adjuvants are supposedly responsible, at least according to some investigators, for the encephalomyelitis

For many reasons the guinea pig offers good material for immunologic, pathologic and early symptomatologic studies These animals

6 Ferraro, A Arch Neurol & Psychiat **52** 443, 1944

7 Freund, J, and McDermott, K Proc Soc Exper Biol & Med **49** 548, 1942

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14 Alvort, E C, Jr Proc Soc Exper Biol & Med **67** 4, 1948

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16 (a) Cazzullo, C L, and Ferraro, A J Neuropath & Exper Neurol **8** 70, 1949 (b) Ferraro, A, and Cazzullo, C L *ibid* **8** 61, 1949

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are less expensive than monkeys, more easily handled and more easily housed in large numbers, and in them the disease appears more systematically. Moreover, in these animals we are able to produce a diffuse encephalomyelitis with a higher rate of morbidity by employing the intraperitoneal route for the introduction of the antigen.

MATERIALS AND METHODS

The animals, carefully selected, were kept under observation for a period of ten days and then divided into lots of various weights, most of them weighing from 450 to 550 Gm.

In all the experiments we used as an antigen homologous brain taken from ether-killed normal guinea pigs, suspended in 12 per cent isotonic sodium chloride solution in a Waring blender and then incorporated into a water-in-oil emulsion. Falba^{18a} and bayol F^{18b} were used as emulsifying agents. A given amount of heat-killed tubercle bacilli was dissolved in a small mortar, and a given amount of bayol was then added (table 1). The tubercle bacilli, obtained through the courtesy

Composition of Emulsion

Materials Injected	Amount per Cubic Centimeter	Amount per Guinea Pig	
		Intramuscularly	Intraperitoneally
Normal guinea pig brain	0.033 Gm	3 cc	1 cc
Sodium chloride	0.4 cc		
Falba ^{18a}	0.2 cc		
Bayol ^{18b}	0.4 cc		
Heat killed tubercle bacilli	0.33 mg		

of Freund, were of a human type, strain Jamaica 22. After emulsification, the mixture was transferred to sterile bottles, covered with rubber caps and stored in the ice box. A sample of every emulsion was checked for sterility with blood agar plates.

Two lots of guinea pigs were used, numbering 10 and 43 animals, respectively. Each animal of the first lot received an injection of 3 cc of emulsion into the muscles of the left and right lateral regions of the neck. A 20 gage, 1 inch (2.5 cm) length needle was used for the injection. Some of the animals temporarily lost their appetite after the injection and decreased in weight within the first twenty-four hours.

Each animal of the second lot received an intraperitoneal injection of 1 cc of the Freund emulsion. For this injection we used a 22 gage, 1 inch length needle, which was inserted into the lower part of the abdomen along the medial line. The guinea pig was kept in a head downward position by the well known device of putting it, head first, into the examiner's pocket. In our experience, acute peritonitis never occurred.

Many reasons induced us to use the intraperitoneal route for the introduction of the antigen. First, we tried to keep the muscles of the neck free for injections

18 (a) Falba[®] is an absorption base derived from hydrous wool fat and composed of a mixture of oxycholesterol and cholesterol. (b) Bayol is a paraffin oil of light viscosity.

of protective substances. Second, we tried to avoid excessive trauma, having received the impression that the intraperitoneal route traumatized the animals very little. Third, we wished to avoid complications due to the slow absorption of the antigen injected intramuscularly. The antigen, which occasionally becomes encapsulated, may be absorbed from time to time, thus influencing the clinical course of the disease. Antigen injected intraperitoneally seems to be absorbed faster as a whole, though one can observe even after long periods a residue of the emulsion in the peritoneal cavity encapsulated in droplike formations of various sizes, some adhering to the various organs.

MORBIDITY AND MORTALITY RATES

The mortality and morbidity following intramuscular injection of the antigen have been reported by others. In the lot of animals of the weight mentioned which received intraperitoneal injections the morbidity was much higher, 39 of the 43 animals contracted encephalomyelitis within the first three weeks after the injection of the antigen.

The mortality was also high. In fact, only 2 of 13 animals of one group survived, 3 of 15 of a second group, and 4 of 15 of a third group. Of the surviving animals of the first group, 1 presented a mild form of the disease, 1 appeared normal. Of the 3 survivors of the second group, 1 suffered from paresis of the posterior limbs and 2 appeared normal. Of the 4 survivors of the third group, 1 was paralyzed, 2 were paretic and 1 may be considered normal. The mortality reached its highest peak within fifteen to twenty-two days.

The appearance of the first symptoms was rapid, and the illness usually followed an acute course. In some cases, following a subacute course, death occurred only after thirty-two to fifty-five days. Some animals not belonging to the series now being reported have lived seventy-eight and seventy-nine days, respectively.

As our experience extends to a larger number of guinea pigs, we feel that a relationship seems to exist between the weight of the animals and the amount of intraperitoneal antigen necessary to precipitate the disease.

SYMPTOMATOLOGIC OBSERVATIONS

Loss of Weight—The study of the weight curve is particularly interesting. By watching the weight and the general nutrition of the animals, one can somewhat anticipate the onset and progress of the disease, a close correlation of the weight curve and the clinical course having been observed in our animals. There occurs, as a general rule, a drop in weight with the appearance of the first symptoms. Sometimes the loss shortly precedes the appearance of the first symptoms, sometimes it starts simultaneously and progresses steadily until the death of the animals. Particularly interesting is the weight curve of the acute form of the disease, in which the drop occurs earlier and progresses very rapidly to the time of death (fig 1). On the other hand, in cases in which the clinical course is protracted, the loss of weight appears with the first symptoms and is most impressive during the phase of aggravation. With the stabilization of the disease the animals regain weight and recover good nutrition. In some cases remission and relapses are respectively accompanied or followed by gain and by loss of weight (fig 2). It is advisable to weigh and examine the animals possibly every day, after feeding.

Related to the weight is the appetite of the animals, which generally decreases shortly before the appearance of the first symptoms. When encephalomyelitis, hemiplegia, ascending paralysis or cerebellovestibular symptoms become fully developed, the animal consumes less food. Convulsive seizures do not seem to affect appetite. Following the period of invasion of the disease, if recovery or improvement sets in, the appetite increases.

Muscular Impairment—Although it is difficult to make semeiologic observations in animals as small as guinea pigs, it has been possible to observe as first symptoms hypotonia and thinning of the muscles of the lumbosacral region. Asthenia is another early finding, which can best be tested by forcing the animal on its back. The righting reflexes are among the very first to be impaired. In advanced cases of asthenia the animal lies on its back exhausted. The asthenia

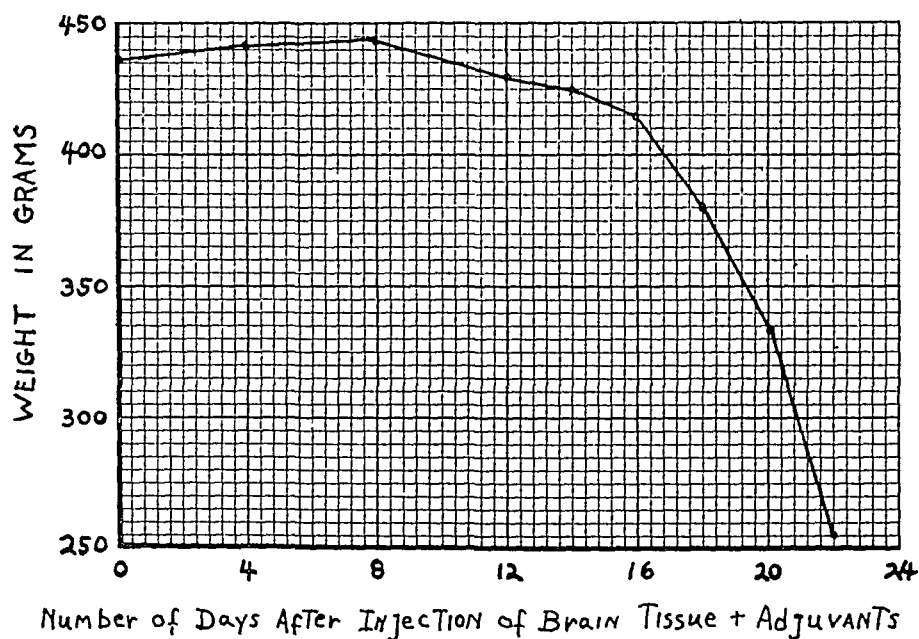


Fig 1—Composite weight curve of guinea pigs dying of acute experimental encephalomyelitis

is followed, or accompanied little by little, by motor involvement related to the localization of the disease. Hypotonia may be present in the absence of asthenia.

Transverse myelitis occurs quite often. In this case the first symptoms are usually hypotonia and thinning of the muscles of the lumbosacral region and of the posterior limbs, deficient righting reflexes and asthenia. Muscular power and motility gradually decrease, and paresis or paralysis of one or two of the posterior extremities sets in, associated with incontinence of feces and urine. Deterioration of the general condition occurs rapidly. Occasionally, the abdomen swells up because of intestinal paralysis, and in certain cases astasia of the trunk and the head and small rhythmic tremors are observed. Trophic changes, such as loss of hair or decubitus, complicate sphincter dysfunctions. Paralysis of the posterior limbs, at first flaccid, later becomes spastic and in extension.

Diffuse encephalomyelitis recalling that of gradual ascending myelitis in human subjects is at times observed, the spinal cord showing the initial invasion and the

involvement progressing rapidly toward the higher centers. In this variety the early symptoms begin also with hypotonia of the muscles of the lumbosacral region, followed by asthenia and paresis, milder in character than the definite flaccid paralysis of the first group. The dysfunction of sphincters appears later and is less pronounced than in the first group. The course of the disease is quite rapid, soon involving the anterior extremities. Here one encounters ataxia and dysmetria and oscillation of major or minor amplitude of the head and trunk. These oscillations may be followed by a rhythmic tremor of the head and the anterior extremities. Subsequently nystagmus occurs, associated at times with symptoms of the cerebellovestibular series.

We never observed paralysis of the tongue or of the muscles of the face, but some difficulty in swallowing was noticed. In the more advanced stage, which is usually reached within a few days, tetraparalysis may develop. Dyspnea sets in and becomes more and more noticeable even at rest, becoming very marked in rest.

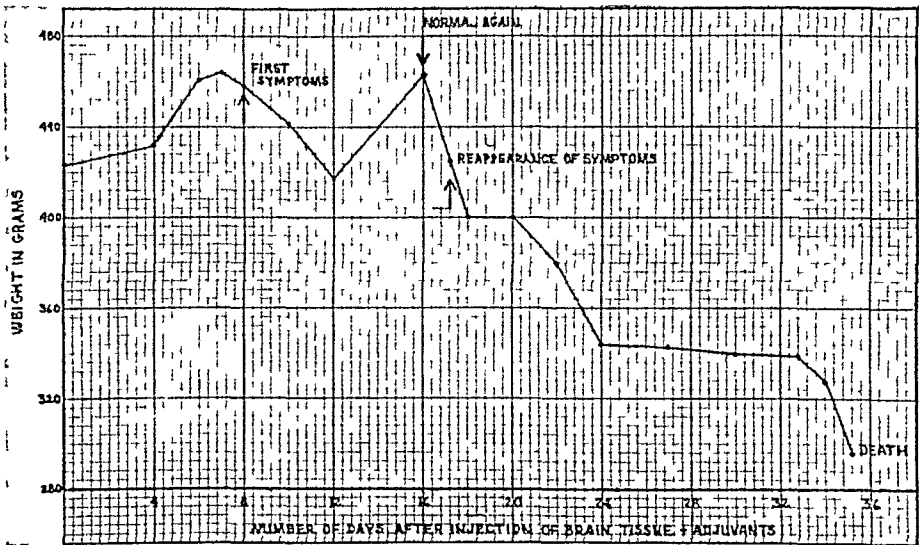


Fig 2—Weight curve showing correlation between weight fluctuation and appearance and disappearance of symptoms, relapse and aggravation of experimental encephalomyelitis

At times generalized convulsions appear. The convulsions usually occur at spaced intervals, but occasionally actual status epilepticus develops. The attacks generally last a few seconds. Some of the attacks are jacksonian in type, becoming generalized later on.

The hemiplegic type is not often encountered. Following the initial general asthenia and hypotonia of the muscles of the lumbosacral region and of the posterior extremities, paresis and then flaccid paralysis of one of the posterior extremities occurs, soon followed by the same occurrence in the homologous anterior extremity. The animal lies on the paralyzed side. Ambulation becomes particularly difficult, but ataxia and tremors are less pronounced. Nystagmus seldom occurs. Localized convulsions, which usually become generalized after some attacks, are at times observed. Transition from hemiparesis to tetraparalysis

is at times encountered, and death usually occurs quite rapidly. The drop in weight is less striking in these cases than in the other two groups.

Of particular interest are the postural changes that occur in a few animals. Some guinea pigs are seen with a twist of head and trunk, so that the back shows a more or less pronounced external convexity, the head and trunk turned more often toward the left. In motion these animals disclose a tendency toward circular walking. The movements are brusque, uncoordinated and at times obviously hypermetric. When the animal is lifted by the trunk and placed again on the ground, it no sooner touches the floor than it resumes its circular posture.

As soon as six or seven days after the intraperitoneal injection, one may note initial neurologic symptoms.

Delayed Paralysis—In a few instances, following intraperitoneal injection of the antigen, only general asthemia and slight hypotonia of the hindlegs develop. These symptoms may even disappear gradually, although the animal may fail to gain weight. Though the animal is not healthy, no definite neurologic symptoms are manifest. It is only after many weeks or sometimes several months that the clinical picture changes and gradually a marked neurologic syndrome develops, leading to paraplegia of the extremities. Such delayed reactions are under investigation.

Remissions—While this report was being prepared, interesting observations were made in a group of larger animals. In these animals, remissions were noted, and, following early asthemia, hypotonia and even paresis of one or both extremities, a gradual recovery was observed. The degree of recovery varied from animal to animal.

In these animals showing remission and improvement or even recovery the related histopathologic changes are presently being investigated.

HISTOPATHOLOGIC CHANGES

In the group of guinea pigs in which encephalomyelitis developed following intramuscular injection of brain emulsion plus adjuvants the histopathologic process is to be considered mainly as an acute inflammatory one.

The distribution of the inflammatory reaction was about the same in all the animals. The cerebrum, the cerebellum and the spinal cord were all involved. In the cerebrum, it was the white matter which was mainly affected, the cortex disclosing generally milder changes. However, the soft meninges all over the cerebrum and the cerebellum disclosed an inflammatory reaction, which in certain areas was moderate and followed the folds of the pia-arachnoid in the depth of the cerebral convolutions and in others was intense, with large numbers of inflammatory cells stratified along, or around, blood vessels.

Of the various portions of the cerebrum, the frontal area seemed the less involved. More pronounced was the involvement of the cornu ammonis, especially along the uncus and in the adjacent areas of the hippocampus, particularly in the subependymal layer of the lateral ventricles.



Fig 3—Various areas in the pons and the medulla with inflammatory reaction. Nissl stain

In the brain stem, scanty pathologic change was found in the mesencephalon, although at times the corpora quadrigemina disclosed marked involvement. In the tegmentum of the pons and in the medulla oblongata more intense inflammatory reaction was seen (fig 3).

In the white matter of the cerebellum patches of inflammatory reaction were often noted, but it was the cerebellar nuclei which constituted an area of predilection for pathologic change.

In the spinal cord the white substance was the seat of the major involvement, the gray matter was involved to a lesser degree. In the meninges, inflammatory cells were often stratified within the pia and along the blood vessels within the septums.

In all the mentioned areas the inflammatory reaction consisted generally in perivascular exudation, the exudate surrounding mostly veins, when the reaction was intense, small and large arteries were also involved.

The exudate consisted of several types of cells: polymorphonuclear elements of the granulocytic series (band cells and segmented cells), lymphocytes, large mononuclear cells, plasmacytes and histiocytes. The polymorphonuclear cells were quite numerous, though not as numerous as the lymphocytes. They were present above all in the areas of more recent involvement and in cases of more recent date. In older lesions the lymphocytes predominated, associated with the large mononuclear elements and the histiocytes. In addition to the histiocytes originating from blood vessels there were histiocytes in the formation of which the microglia and oligodendroglia cells had participated.

In our material the plasmacytes were not as numerous as reported by others. Only here and there were we able to detect them with the Nissl stain. It is possible, however, that with more specific stains for plasmacytes one might detect a larger number of them.

Large mononuclear cells were more commonly encountered in cases in which the animals had survived long periods.

The major contribution to the histiocytic reaction was furnished by the microglia cells. These cells were seen even with the Nissl stain as participating in the exudation, partly mixed with other cells and partly surrounding the layer of lymphocytes (fig 4). Specific impregnation with the Hortega method has not been very successful in our material and only fragments of the histologic picture are available. Compound granule cells derived from microglia cells seemed less numerous than large mononuclear cells.

We failed to find definite correlation between the age of the pathologic process and the type of the perivascular exudate. This is due in our opinion to the fact that in the course of a long-standing process, leading to chronic lesions, new showers of the cells characterizing the acute reaction occur with renewed output of antigen from the site of

injection, as a result of which the process is often a mixed one—acute, subacute and chronic. The life span of the animals surviving outstanding neurologic symptoms has not been long, and further investigations are necessary to solve this problem.

In 2 animals which survived the onset of the paralysis several weeks, we found that the inflammatory reaction involving both brain and spinal cord was mild in one and severe and diffuse in the other.

The same comment applies to the reaction of the glia cells. We refer particularly to the astrocytes, which in the early stages disclosed acute regressive changes in the midst or in the immediate vicinity of

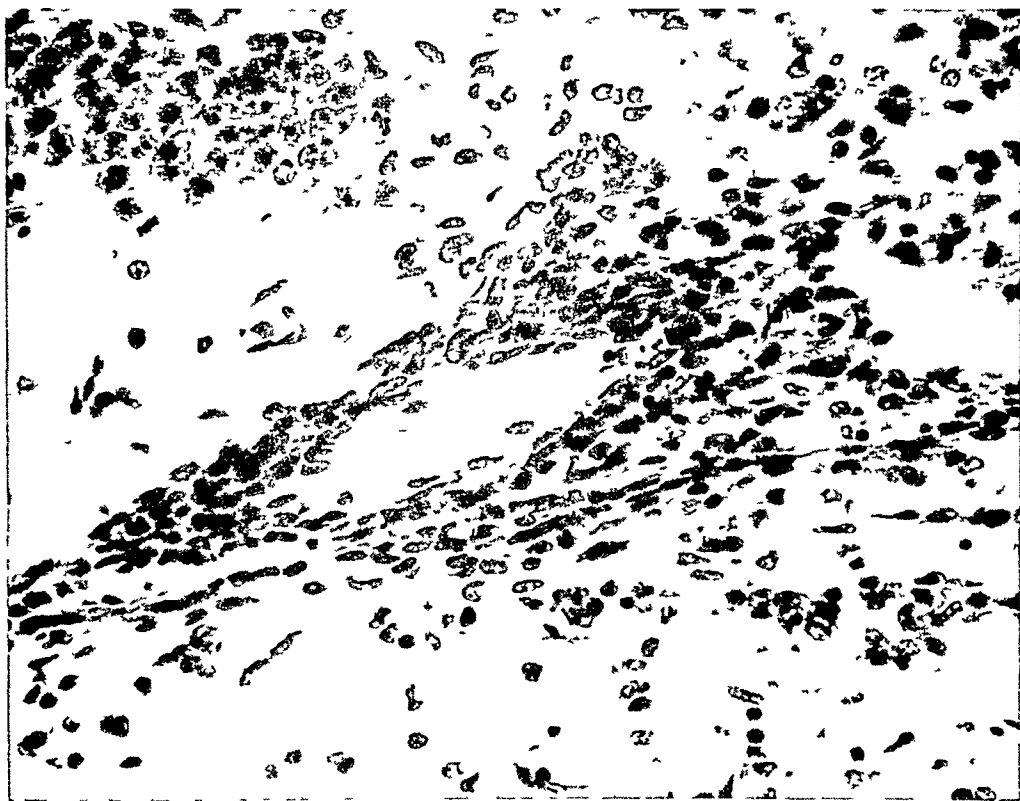


Fig 4—Microglial reactions surrounding a blood vessel. Lymphocytes are intermingled with these cells. Nissl stain.

an inflammatory area. Later on, a distinct type of progressive reaction occurred. It consisted of hypertrophy of astrocytes, visible especially in animals which had survived several weeks with neurologic symptoms (fig 5). In the spinal cord, where often the inflammatory lesions were more pronounced, one failed to observe such a progressive reaction. This applies particularly to the gray matter where one got the impression that irrespective of the duration of the disease a regressive change of the astrocytes, clasmotondendrosis, was prevalent. In the white substance a better attempt at progressive reaction was noticeable,

never, however, reaching the intensity detected in the cerebral white matter

The reaction of the blood vessels in the involved area varied from case to case. Any relationship existing between the reaction of the blood vessel walls and the severity of the inflammatory process was not definite. Numerous blood vessels disclosed swelling of the endothelial lining cells. Some disclosed a thickening of all layers of the wall, with predominance at times in the adventitia, the media or the intima. Often one noticed a deformity of the intima resulting from

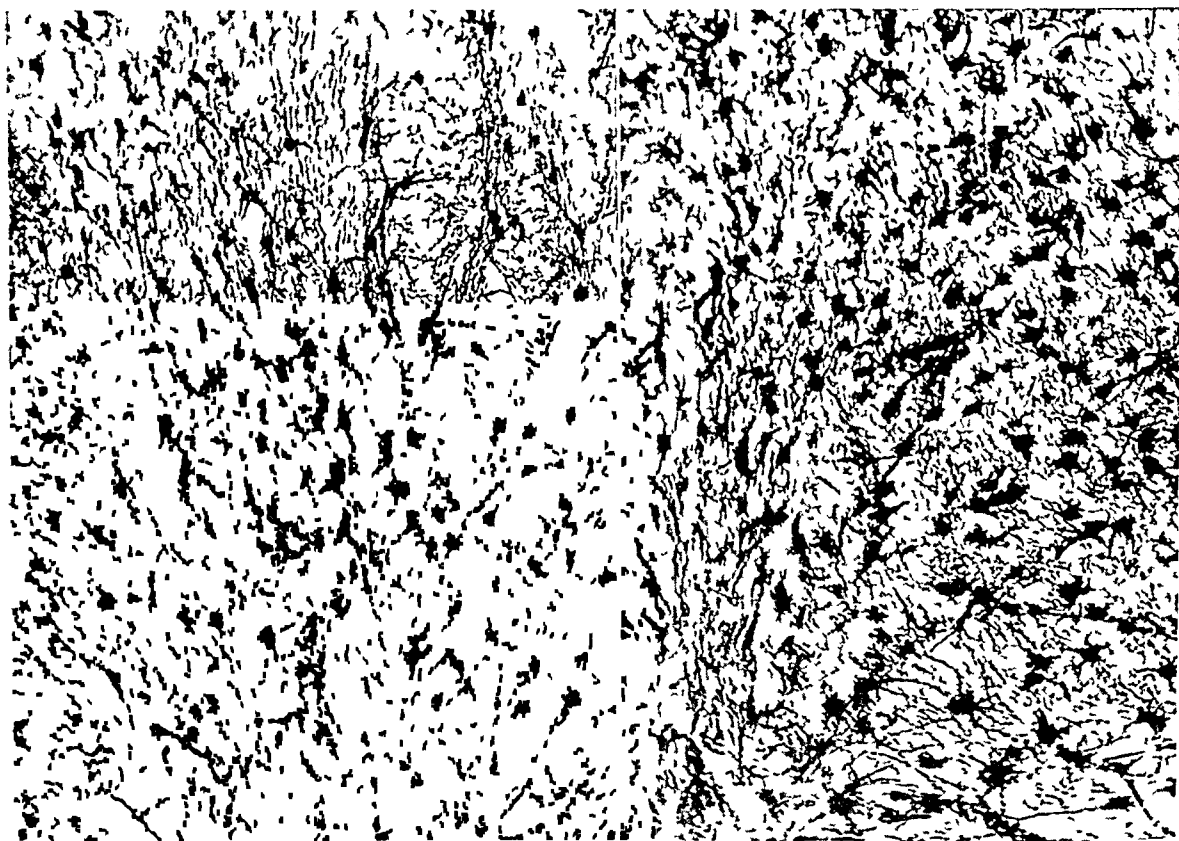


Fig 5—Progressive changes of astrocytes (hypertrophy and hyperplasia) in the white substance. Cajal gold chloride-mercuric chloride method

unequal thickening and folding of the intimal layer. At times the thickening of the intima and the intramural and perivascular reaction was of such intensity that partial or total occlusion of the blood vessel lumen occurred. No fresh thrombi were detected with the Nissl or the hematoxylin-eosin stain.

Myelin sheath and axis-cylinder preparations disclosed a moderate amount of change. Generally speaking, we have failed to find the typical areas of demyelination reported occurring in monkeys in the same type of encephalomyelitis. Here and there in spreading areas

of inflammatory reaction only rarefaction and occasionally destruction of myelin sheaths were visible

The axis-cylinders, particularly those of the spinal cord, disclosed, in relation with a more severe type of inflammatory reaction, various degrees of swelling and fragmentation

In the group of guinea pigs in which encephalomyelitis followed intraperitoneal injection of brain emulsion plus adjuvants, the fundamental pathologic process in both brain and spinal cord was substantially identical with the one occurring in animals whose encephalomyelitis followed intramuscular injections

In the brain, particularly, most of the pathologic features were identical as to type, distribution and intensity of the process (a) Type A diffuse meningeal reaction and perivascular reaction extended to numerous areas of the brain, with predilection for the white substance. The meningeal exudate, especially in the later stages, was mainly formed by lymphocytes, large mononuclear cells and numerous chromatophores. There the perivascular exudate was also predominantly formed of lymphocytes and large mononuclear cells. In less advanced stages, in guinea pigs which survived only a few days after the onset of the first neurologic symptoms, polymorphonuclear cells were also present and plasmacytes were occasionally found. As with the intramuscular way, no giant cells were detected

(b) Distribution. The distribution of the perivascular reaction followed in this group the same pattern reported in the first group, i. e., predilection for the periventricular areas, the diencephalon, the medulla and particularly the vestibular nuclei and the nuclei of the cerebellum proper

(c) Intensity. The intensity of the pathologic reaction varied from case to case, bearing no strict relationship to the length of survival of the animals

In the spinal cord, however, there seemed to be some variants of the changes resulting from the intramuscular injections. The difference was in both intensity and type of perivascular reaction. We feel that the process in the spinal cord was more severe following intraperitoneal injection of antigen. Most of the white columns of the spinal cord appeared severely involved. In one case severe involvement was present after a survival of fifty-two days whereas in another the same severe involvement was present only seven days after the onset of the first symptoms

Not only a quantitative but also a qualitative difference is appreciated in the sense that surrounding the blood vessels mostly lymphocytes and large mononuclear cells were seen. The number of inflammatory cells and their compactness were such that the involved

blood vessels recalled the appearance of periarteritis nodosa. Though no necrotic changes were seen, higher power magnification brought out more clearly the abnormal thickening of the blood vessel walls,



Fig 6—Severe perivascular reaction in lateral columns. Nissl stain.

leading in certain instances to complete mechanical occlusion of the blood vessel's lumen (fig 6).

Myelin sheath stains pointed to generally well preserved myelin sheath in both cerebrum and cerebellum. The glia reaction did not differ from that resulting from intramuscular injection of antigen. The same applies to the axis-cylinders.

In the two groups in which encephalomyelitis resulted from intramuscular and intraperitoneal injections of antigen, respectively, hemorrhages and necrosis were not outstanding. Nothing comparable to the Aithus phenomenon has ever been observed in our material. Occasional necrosis has been detected in the white matter of the spinal cord and only occasional fibrinoid necrosis of the blood vessel walls.

Concerning the reaction of other parenchymas, lung, liver, spleen, etc., we have drawn from our material interesting pathologic data contrasting with the negative results of other investigators. A detailed report will form the object of a separate communication.

Relationship of the Encephalomyelitis Produced in Guinea Pigs and That Produced in Monkeys—It is important to raise this question in view of the fact that in guinea pigs the demyelination was not an essential part of the histopathologic process. Because of this variation, some investigators might feel that the two processes are different. Our contention is that the encephalomyelitis of guinea pigs produced with the same antigen used for monkeys and as a result of the same pathogenic mechanism is identical with the encephalomyelitis experimentally induced in monkeys.

Not all animals react in the same manner to the same etiopathogenetic factors. Dogs react with endarteritis proliferans to lead poisoning—cats do not. Dogs react with softening of the globus pallidus to carbon monoxide poisoning—rabbits do not. Let us not forget that next to the chapter of general pathology there is the chapter of comparative pathology, which is still to be fully investigated in animals.

It is important to establish the identity of the process in both guinea pigs and monkeys because of the fact that in the progress of our investigation we need animals that are easily handled, relatively inexpensive and easily housed in large numbers. In our further immunologic studies and studies to assay various methods for the prevention of the disease, we want to feel that we are dealing in guinea pigs with the same fundamental pathologic process which in monkeys is associated with demyelination.

SUMMARY

Emulsion of 3 cc of normal brain plus adjuvants, according to Freund's technic, introduced intramuscularly produces diffuse encephalomyelitis in guinea pigs, 1 cc of the same emulsion introduced intraperitoneally produces the same type of encephalomyelitis. The

reasons for using this route are discussed. Symptomatically, the disease involves the whole nervous system. Initial symptoms are hypotonia and asthenia, evidenced by poor righting reflexes. Ataxia, paresis, paralysis, tremors and convulsions frequently develop. The paralysis of the posterior limbs is flaccid, becoming spastic later on. The lesions are diffuse, but one can find a sort of predilection for the spinal cord, the brain stem and the cerebellum. Variations in the weight and the nutrition of the animals are discussed. Histologically, the main feature is a perivascular inflammatory reaction of the brain and spinal cord, an expression of allergic encephalomyelitis. Demyelination is scarce whereas progressive glia reaction is encountered. Hemorrhages and necrosis are scanty. The clinical and the pathologic features of this encephalomyelitis bear close similarities to the encephalomyelitis induced by the same procedure in monkeys.

SKELETAL GROWTH AND DEVELOPMENT IN MICE FED A HIGH PROTEIN DIET

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THE NUTRITIONAL requirements for growth and maintenance have been extensively studied by withdrawing certain constituents from the diet. However, less attention has been paid to the effect of feeding excessive amounts of such basic components as fat, protein or carbohydrate. Data concerning weights and chemical analyses of the carcass are available, whereas reports of histologic changes in the skeleton are lacking.

In continuation of our investigations of the role of nutritional factors in skeletal growth and aging we have analyzed the course of these processes in young mice fed a diet high in casein. The results obtained will presently be described.

MATERIAL AND METHODS

Fifty-two virgin female mice of the inbred strain C57 black raised in our laboratory were used. At the age of 4 weeks, the animals, weighing then about 10 Gm, were divided into two groups.

Series 1—Twenty-six mice were fed a stock diet of a commercial chow¹ which contains the following ingredients

	Per Cent
Moisture	8.90
Protein	26.18
Fat	5.35
Fiber	4.62
Ash	6.49
Nitrogen-free extract	48.46
Calcium	1.17
Phosphorus	0.87

From the Snodgras Laboratory of Pathology, City Hospital, and the Department of Pathology, Washington University School of Medicine.

This investigation was supported by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council and by a grant from the Committee on Scientific Research of the American Medical Association.

¹ The chow used was B-2362 made by the Ralston Purina Company, St. Louis. Data concerning the mineral and vitamin contents can be found in the pamphlet issued by the company.

Series 2—Twenty-six mice received a diet high in casein and of the following composition

	Per Cent
Crude casein	52.69
Crude fat	2.64
Crude fiber	2.32
Nitrogen-free extract	29.49
Ash	3.96
Calcium	1.24
Phosphorus	0.70

An adequate vitamin content was assured by the use of 4 per cent brewers' dried yeast, 4 per cent dehydrated alfalfa meal and a special vitamin premix containing vitamin D, riboflavin, thiamine and nicotinic acid²

This ration was given to the animals as a finely ground meal in unlimited amounts and was readily eaten. Water was likewise given *ad libitum*. Usually

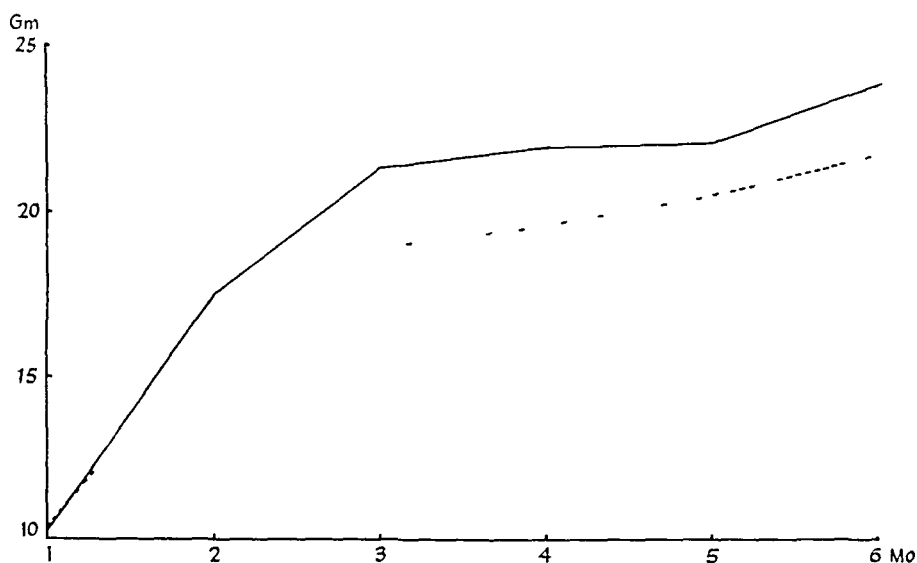


Fig 1—Mean weight curves of mice fed the stock ration (—) and mice fed the high casein ration (---)

6 animals were kept in one enamel pan measuring $12\frac{1}{2}$ by $8\frac{1}{2}$ by $4\frac{1}{2}$ inches (about 32 by 21.5 by 11.5 cm). Weights were taken once a week up to the age of 4 months, thereafter the mice were weighed at monthly intervals. The animals were killed in the following order: Four mice of each group at the ages of 6 weeks or 2 months, and 6 mice of each group at the ages of 3, 4 or 6 months, respectively.

At necropsy the individual weights were recorded, the tibia, the femur and the knee joint were removed as a whole, vertebrae and pieces of internal and endocrine organs were secured and fixed in 4 per cent formaldehyde solution. The bones were decalcified in 5 per cent nitric acid, neutralized in 5 per cent alum (aluminum and potassium sulfate) and embedded in paraffin. Semiserial sections were prepared and stained with hematoxylin and eosin for microscopic examination.

² The Ralston Purina Company cooperated in the preparation of this ration.

EFFECT ON WEIGHT

As seen from figure 1, the animals kept on the high casein diet gained weight steadily but did so more slowly than those receiving the stock ration

The table shows the mean weights and the maximum and minimum deviations

The individual weights of the mice fed the stock diet showed considerable variation, however, there were but slight differences in the individual weights of the animals receiving the high protein ration

HISTOLOGIC OBSERVATIONS

The description of the tissue changes is based on observation of the growth zone at the upper end of the tibia

Series 1 Animals 6 Weeks Old—In mice fed the stock ration, the growth zones were about 200 microns wide (fig 2A) The cartilage cell rows showed

The Mean Weights (in Grams) and the Maximum and Minimum Deviations

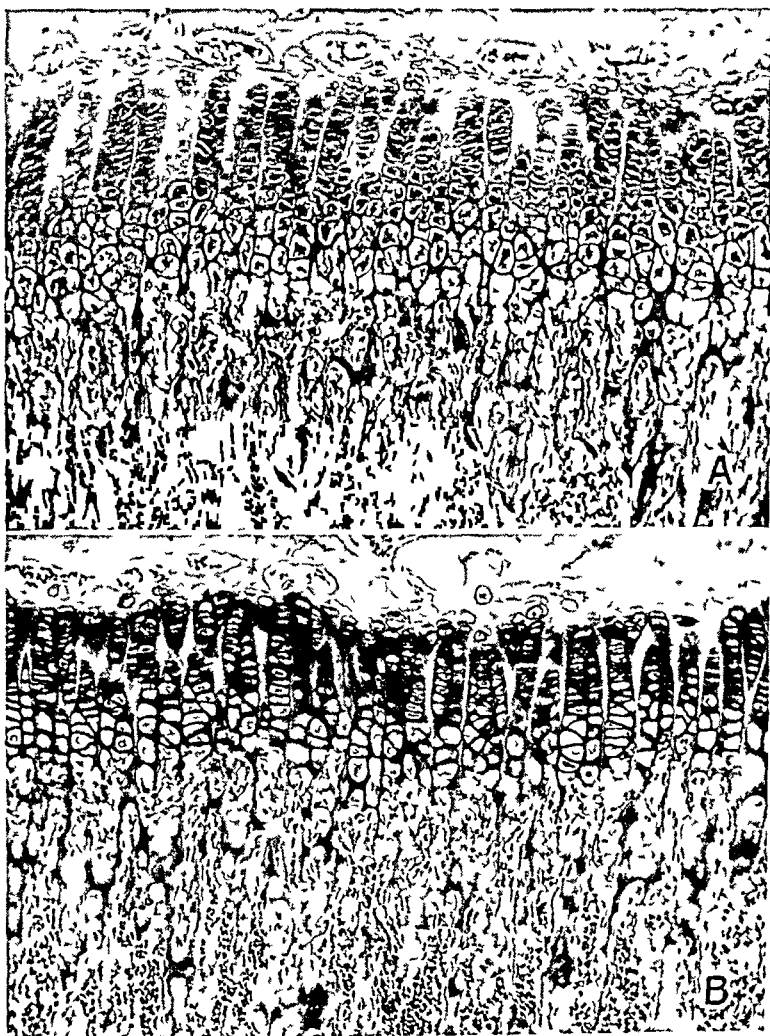
Age of Animals, Mo	Animals Fed the Stock Diet	Animals Fed the High Casein Diet
1 (initial)	10 2 { maximum 0 8 minimum 0 2	10 4 { maximum 1 1 minimum 0 4
2	17 5 { maximum 0 5 minimum 2 5	16 6 { maximum 0 4 minimum 0 6
3	21 3 { maximum 1 7 minimum 1 3	18 9 { maximum 0 1 minimum 0 9
4	21 9 { maximum 2 1 minimum 1 9	19 6 { maximum 0 4 minimum 0 6
5	22 1 { maximum 1 9 minimum 2 1	20 5 { maximum 0 5 minimum 0 5
6	23 9 { maximum 2 1 minimum 2 9	21 8 { maximum 0 2 minimum 0 8

regular configuration and were composed of 8 to 10 small columnar and 3 or 4 hypertrophic cells Here and there, mitotic figures were seen in the upper layers of the cartilage columns Thin layers of chondromucoid ground substance separated the individual cartilage cell rows from one another The metaphysis was vascular, the primary spongiosa was represented by thin, short trabeculae The shaft was composed of large osteocytes and abundant interstitial substance The articular cartilage contained two layers of small spindle-shaped cells in the sliding zone, the resting cartilage cells were being converted into proliferating and hypertrophic cells

In mice fed the high casein diet for two weeks, the growth zones measured an average of 140 microns (fig 2B) The cartilage showed the regular structure and increased calcification of the matrix In the individual cartilage cell row 6 to 8 columnar and 2 or 3 hypertrophic cells were counted Active growth was indicated by the numerous mitotic figures appearing in the columnar cartilage cells The latter were larger, and the breakdown of the hypertrophic cartilage cells occurred farther proximally than ordinarily The primary spicules were more numerous, thicker and longer than in the animals receiving the stock ration The

osteocytes of the shaft were small, and the bony ground substance was markedly calcified. The articular cartilage was represented by fewer undifferentiated and more hypertrophic cells, and bone replacement of cartilage was farther advanced than in the mice kept on the stock diet.

Series 2 Animals 2 Months Old—In mice receiving the stock diet, the growth zones were about 125 microns wide (fig 3A). The cartilaginous matrix was more



Figures 2 to 5 show sections through the epiphyseal growth zones at the upper ends of tibias of virgin female mice of strain C57 black, $\times 112$.

Fig 2—*A*, 6 week old mouse fed the stock diet. The growth zone and the metaphysis show the usual structure.

B, 6 week old mouse fed the high casein diet for two weeks. As compared with *A* the growth zone is narrowed, the columnar cartilage cells are larger, and there is more bone in the metaphysis.

abundant than at the age of 6 weeks, and small wedges of hyaline material were noted between the cartilage cell rows. The latter were composed of 5 to 8 columnar and 2 to 3 hypertrophic cells. The metaphyseal trabeculae were slender

and short and covered by continuous layers of large osteoblasts. The appearance of the shaft and the articular tissues was similar to that noted at the age of 6 weeks.

In mice kept on the high casein diet for 1 month, the growth zones were intensely calcified and varied in width from 125 to 140 microns (fig 3B). The individual cartilage cell rows contained 5 to 7 large columnar and, at best, one

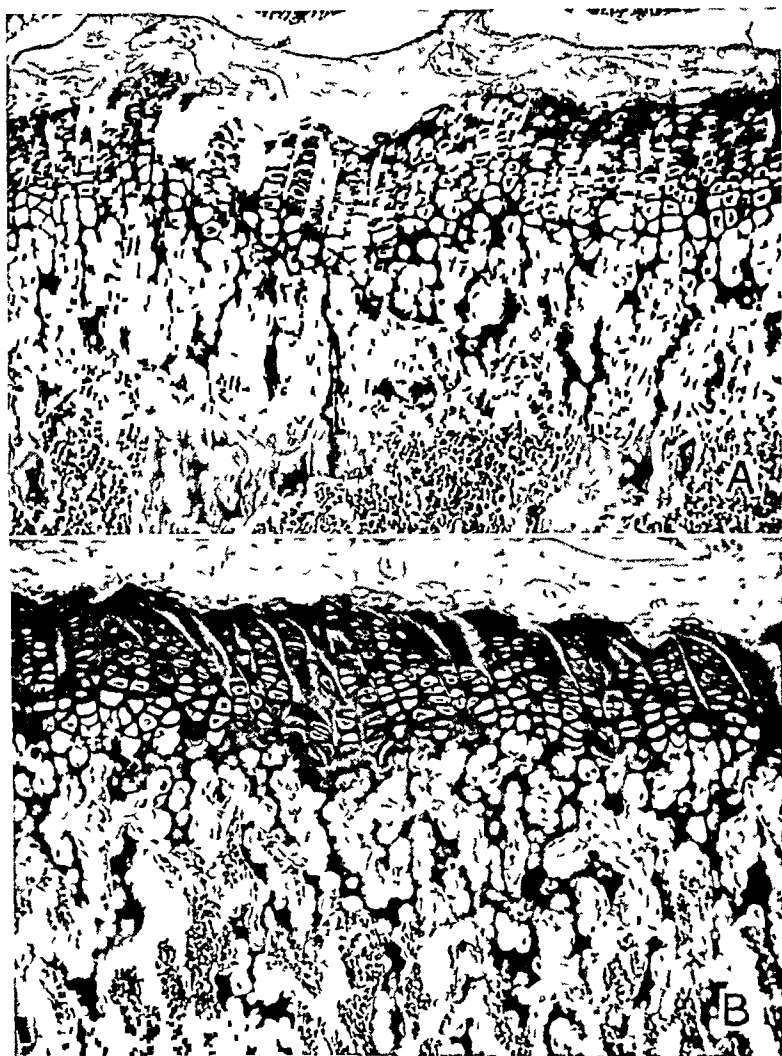


Fig 3—A, 2 month old mouse fed the stock diet. The cartilaginous matrix is more abundant and the trabeculae are shorter and more compact than in the younger animal (fig 2A).

B, 2 month old mouse fed the high casein diet for one month. The growth zone is irregular in width, the columnar cartilage cells are larger, the hypertrophic cells are fewer, and the metaphysial spicules are longer and thicker than those of the control animal (fig 3A).

fully developed hypertrophic cell. Again, the columnar cells showed many mitotic figures and began to enlarge in the more proximal parts of the growth zones. In the metaphysis were numerous short, broad-based spicules, some forming transverse bridges. The matrix of the shaft contained thick, distinct calcium lines. The

articular cartilage was not remarkable. Much subchondral bone had been laid down.

Series 3 Animals 3 Months Old—In the mice receiving the stock diet, the average width of the growth zones was 75 microns. The cartilage cell columns could still be recognized, however, large wedges of dense hyaline ground substance separated the cartilage cell rows from one another or began to replace them. The cartilage columns were shorter than at the earlier ages and composed of 5 to 7 columnar and 2 or 3 hypertrophic cells. The vascularization of the metaphysis was decreased, the spicules contained larger amounts of calcium, they were short, covered by small osteoblasts and connected with one another by bony links. The shaft was composed of small osteocytes and heavily calcified matrix. The articular tissues had not changed as compared with those of the younger age group.

In mice fed the high casein diet for two months, the growth zones were about 80 microns wide. The cartilaginous ground substance had slightly increased in amount. The individual cartilage cell rows were composed of 6 to 8 columnar and 2 or 3 hypertrophic cells. The cartilage cells proliferated actively, as was indicated by the presence of many mitotic figures. The hypertrophy of the columnar cells, however, was now less conspicuous than at the earlier ages, these cells enlarged only after they had come to lie near the distal end of the cell rows. Fully developed hypertrophic cells were more numerous than before. The vascularization of the metaphysis was good, many spicules were seen in the subchondral layer. The cortex of the shaft and the articular tissues were not remarkable.

Series 4 Animals 4 Months Old—In mice fed the stock ration, the growth zones were narrow and had become irregular in width (fig 4A). Much hyalinized intercellular substance was present. The cartilage cell rows contained 4 or 5 columnar and 1 or 2 small cells of hypertrophic type. The proliferation of the columnar cartilage cells was at a low or had come to a standstill. Single cartilage cells or whole cartilage cell rows had broken down, and plugs of amorphous material had taken the place of destroyed cartilage. These plugs were thick and frequently showed horizontal cracks. While some bony trabeculae were still present, the epiphyseal cartilage was separated from the metaphysis by a continuous thick transverse osseous lamella. The cortex of the shaft had increased in thickness. The articular cartilage was in a resting condition, the ligaments and the synovials were not unusual.

In mice kept on the high casein diet for three months (fig 4B), the width of the growth zones was about 80 microns. The individual cartilage cell rows were composed of 5 columnar and 2 fully developed hypertrophic cells. Here and there mitotic proliferation of the columnar cells was observed. The hyalinization of the matrix and the regressive alteration of the cartilage cells were less advanced than in the animals fed the stock ration. Metaphyseal trabeculae were numerous and showed horizontal bridges. In only a few instances a thin and discontinuous bony lamella was seen underneath the epiphyseal cartilage. The compacta of the shaft was not remarkable. The articular cartilage cells were large. In 2 of the 6 animals of this group the cartilage of the intermediate zone had undergone hypertrophy, in addition, a number of cell nuclei showed pyknosis or karyolysis. These degenerating cells were surrounded by a rim of basophilic matrix. In 2 other animals the collagenous tissue of the ligaments was loosened and had undergone mucoid change.

Series 5 Animals 6 Months Old—In mice fed the stock diet, the zones of growth consisted of a narrow plate of inactive, sclerosed and hyalinized cartilage

(fig 5A) Numerous cartilage cell rows had been destroyed, and many thick, amorphous, partly calcified or ossified plugs traversed the epiphysal disks. There was no longer any difference between the cells of columnar and hypertrophic type. The inactive epiphysal plate was sealed off from the metaphysis by a thick continuous bony lamella, indicating that epiphysal growth had ceased altogether. Here and there, a bony spicule was seen in the metaphysis. There was no indication

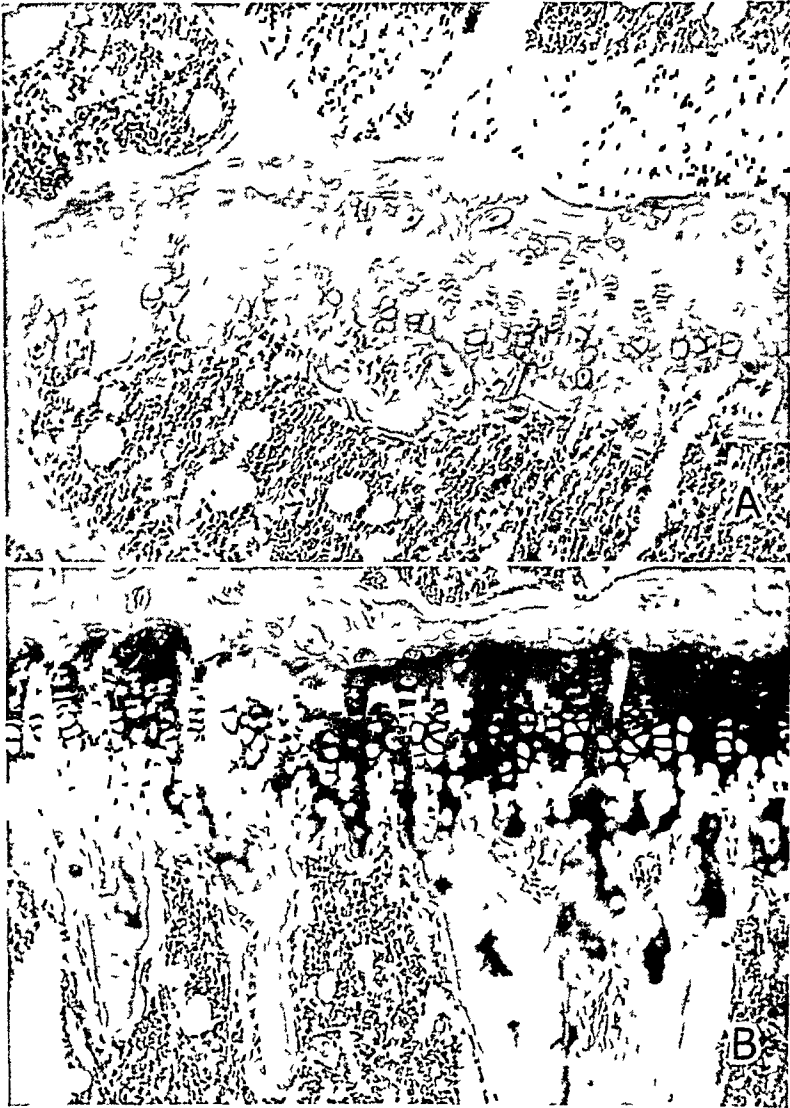


Fig 4—*A*, 4 month old mouse kept on the stock diet. There is marked hyalinization of the cartilage, a transverse bony plate is forming underneath the cartilage, most of the primary spongiosa has been resorbed.

B, 4 month old mouse fed the high casein diet for three months. The columnar arrangement of the epiphysal cartilage is better preserved and hyalinization is less advanced than in the control animal (*A*). The primary spongiosa is still present and represented by thick, long trabeculae, the transverse osseous plate, seen in *A*, has not yet formed.

of an actual or impending break-through suggestive of beginning epiphysiodiaphysal union. The cortical bone was thick and dense. The articular cartilage was

inactive except in 1 animal in which a slight hypertrophy of the cells was noticeable. Neither the synovialis nor the ligaments showed pathologic change.

In mice fed the high casein diet for five months, the growth zones were represented by cartilaginous plates showing but little activity and composed of rows of 4 or 5 columnar cells and 1 or 2 small cells of hypertrophic type (fig 5B). The amorphous plugs of degenerated cartilage were more numerous than at the earlier age but not always quite as large and cracked as in the control animals.

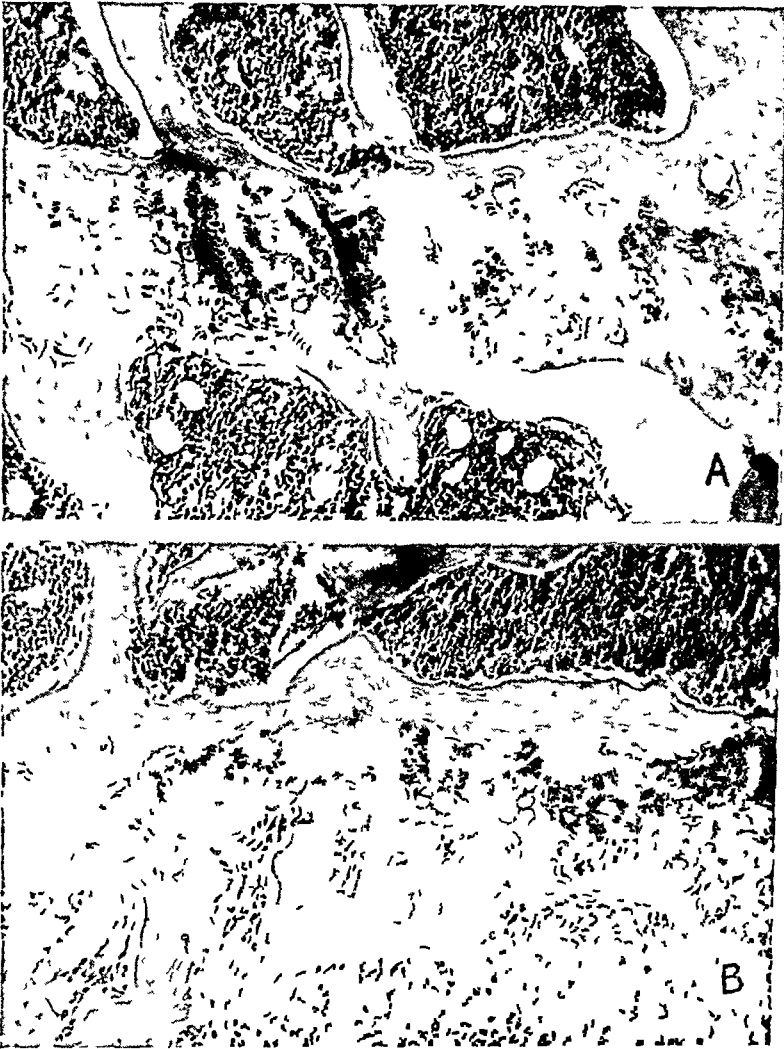


Fig 5—A, 6 month old mouse fed the stock diet. Growth of cartilage has ceased, some bone replacement of cartilage is still going on (left side of photograph). Many cell rows have been replaced by plugs of amorphous material, which show transverse cracks. To the right the cartilage is sealed off from the diaphysal cavity by a solid lamella of bone.

B, 6 month old mouse fed the high casein diet for five months. The structure of the growth zone begins to resemble that of the control animal (A). However, the plugs of degenerated cartilage are not as well defined as in the latter, bone replacement of cartilage is in progress everywhere, and the cartilage has not yet been sealed off from the diaphysal bone marrow.

The subchondral osseous plate was discontinuous, and bone replacement of cartilage was still in progress. The cortex of the shaft contained small and

dense osteocytes. Of the 6 mice of this series, 1 showed moderate hyperplasia and hypertrophy of the articular cartilage, and 2 others swelling and fibrinoid change of the ligaments (fig 6*A*). The synovialis was hyperplastic and penetrated the articular surface, following enlarged preformed vascular channels, causing focal demineralization of bone and replacement of some of the epiphysial marrow (fig 6*B*).

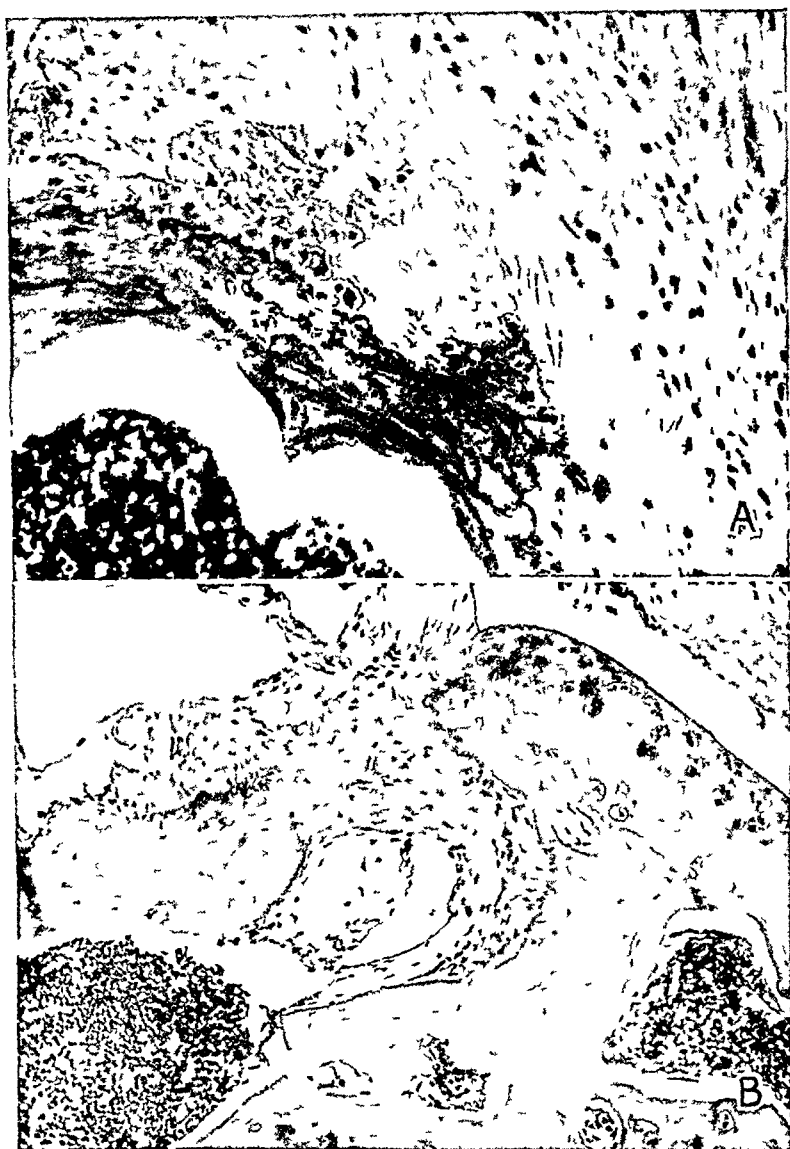


Fig 6—*A* and *B* represent sections through the tibial surfaces of knee joints of 6 month old virgin female mice of strain C57 black kept on the high casein diet for five months $\times 200$. In *A* there is an area of fibrinoid change in one of the cruciate ligaments near its insertion. In *B* synovial tissue penetrates the articular surface and replaces some of the epiphysial bone marrow.

COMMENT

In virgin female mice of strain C57 black fed a stock diet containing 26.18 per cent protein, the growth of the tibia and the femur pro-

ceeded until the age of 4 months. From the third month of life on, regressive processes developing in the epiphysial cartilage increased until they outbalanced those of growth, a state characteristic of the second phase of the skeletal time curve.³

In virgin females of the same strain fed a high casein diet (52.69 per cent) the development of the epiphysial cartilage was accelerated, and bone formation intensified during the early period of epiphysial growth. These findings are in agreement with observations of accelerated body growth of rats receiving a high protein diet.⁴ However, in our mice the onset and progress of regressive changes in the epiphysial cartilage were not hastened in proportion to the intensification of the growth processes. At 4 months of age the growth zones of mice reared on the high casein diet appeared more youthful than those of the animals receiving the stock ration. At the age of 6 months, when the present experiments were terminated, the differences between the control and the experimental groups were less conspicuous, although in some animals receiving the high casein ration the cartilage was still better preserved than in the control series. Whether or not this initial delay of degenerative processes might, at a later age, be compensated by an acceleration and intensification of age changes will have to be decided by long range experiments. Enhanced skeletal aging following initial retardation has been observed in mice and guinea pigs subsequent to ovariectomy.⁵

The mechanism through which the dietary protein acts on the cartilage is unknown. In rats fed a protein-enriched ration there was an absolute and relative decrease of the calcium content of the carcass. This loss could be prevented by adding calcium to the diet.⁵ The calcium requirements of the mouse are relatively low, and the average diet usually contains more than adequate amounts of this mineral. It is thus difficult to produce a calcium deficiency in mice. Still, the slower aging of the epiphysial cartilage might be correlated with decreased deposition of lime salts, although there was no histologic evidence of generalized demineralization of the bone itself.

While excessive dietary casein supplied during the growth period may be utilized for skeletal growth and development, this protein did not exert an early injurious effect on the epiphysial and articular cartilages as did excessive amounts of dietary fat.⁶ In animals fed the high casein diet the articular tissues showed but small foci of hyperplastic cartilage, an occasional degenerated cartilage cell, localized fibrinoid change in a ligament and a slight mucoid degeneration or proliferation of the syno-

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vialis In particular, the changes in ligaments and synovialis were found without or with negligible involvement of the cartilage Conversely, in mice fed the high fat diet the regressive changes predominated There were advanced degeneration, hyperplasia and hypertrophy of the articular tissues, and the synovial reaction occurred in association with severe alterations of the cartilage Whether or not the slight synovial changes found after casein feeding will become intensified after prolonged ingestion of the high protein ration, and whether or not additional changes will develop in the articular tissues under these conditions, remains to be seen Possibly several types of degenerative lesions of joints may occur in the mouse differing in pathogenesis but leading, in the end, to more or less uniform morphologic manifestations

The role of sex in the skeletal response to nutritional factors has yet to be considered Sex differences were found in the body growth of rats receiving a high casein diet, the males gaining weight more rapidly than the females⁴ Since the male is genetically larger than the female, excessive protein may be better utilized by the male in the building up of the skeleton Male mice fed a high casein diet (unpublished data) likewise gained weight more rapidly than females, whose weights in spite of good skeletal growth remained about 10 per cent below those of their controls The early degenerative lesions of joints following the feeding of a high fat diet were noted in male mice The lack of more severe articular changes in the females kept on the high casein ration may be partly attributable to the influence of hormonal factors In females the regular cyclic output of estrogenic hormones increases the density of the cartilage and thus renders it less vulnerable to injurious influences Investigations are in progress to test this assumption

SUMMARY

In growing female mice of strain C57 black, a diet containing 52.69 per cent casein accelerated the growth and development of the epiphysial cartilage and increased bone formation The onset and progress of the regressive processes that occur in the cartilage were temporarily delayed At the age of 6 months the age changes observed in the skeletons of mice fed the high casein ration were of about the same order as those seen in mice kept on the stock diet Only minor degenerative alterations of the articular tissues and slight proliferation of the synovialis occurred under the influence of the high casein diet

THE HUMAN AORTA

Sulfate-Containing Polyuronides and the Deposition of Cholesterol

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THE CONDITIONS under which cholesterol is deposited in the human aorta and in tissues attacked by hereditary xanthomatosis have been studied in previous papers¹ It was shown that besides the cholesterol available from the serum there must exist a tissue factor which is of importance in determining the localization of the deposits

Cholesterol is present in the body as a necessary constituent of all cells Larger amounts are found in certain cellular systems, in liver and in nerve tissue This cholesterol, however, is intracellular and will follow the rules for the individual cell type

In addition there exists, scattered through the organism, a series of cholesterol deposits which have such features in common that a joint treatment should be justified This cholesterol is mainly extracellular If intracellular, it is found in foam cells, indicating that the deposition was primarily extracellular These deposits all increase with advancing age, and occur earlier when the cholesterol content of the serum is increased Table 1 presents a survey of these deposits

As shown in table 1, these tissues have another common feature All show metachromasia when treated with toluidine blue and this metachromasia is ethanol resistant The metachromasia can be present normally (cornea, cartilage, aorta) or as a result of morbid changes (inflammatory conditions, experimental atheromatosis) This metachromasia must, according to Lison,² be assumed to show the presence of polymerized carbohydrate-sulfuric acid esters heparin, chondroitin-sulfuric acid, hyaluronic acid-sulfate From several of the tissues mentioned one of these substances has been isolated, in others they have been shown by histologic studies only

Cholesterol deposits of this type are found only where ethanol-resistant metachromasia is present, and, conversely, where this metachromasia is present, such a deposition is to be expected Hence it is

This study was aided by a grant from the P Carl Petersen Foundation

From the Copenhagen County Hospital Medical Department F and the Health Insurance Physicians Laboratory, Copenhagen, Denmark

1 Faber, M (a) *Acta med Scandinav* **124** 545, 1946, (b) **125** 210, 1946

2 Lison L *Histochimie animale*, Paris, Gauthier-Villars, 1936

justified to propose as a working hypothesis that the presence of ethanol-resistant metachromasia will be the histologic evidence for the

TABLE 1—*Cholesterol Deposits*

Localization	Serum Cholesterol Level During the Deposition	Metachromasia or Known Ester Sulfate	Comments on the Nature of the Deposition of Lipid
Normal Changes in Man			
Cartilage	Normal	Chondroitin-sulfuric acid (Mörner, K Skandinav Arch f Physiol 1 210, 1889)	Rising amounts of cholesterol with age, ⁴ at first below the perichondrium, later also around the enchondral vascularization (Schultz cited by Burger and Schloimka ⁴)
Arcus senilis corneae	Normal and increased	Hyaluronic acid sulfate (Meyer, K, and Chaffee, E Am J Ophth 23: 1320, 1940)	Increasing clinical frequency with age, occurs earlier in hypercholesteremia ^{1b}
Normal aorta	Normal and increased	Chondroitin-sulfuric acid ⁹	Increasing with age, occurs earlier and is more pronounced in hypercholesteremia ^{1b}
Pathologic Changes in Man			
Hypertensive aorta	Normal and increased	Chondroitin-sulfuric acid	More pronounced depositions than in the normal aorta (Björnsson, J Arteriosclerosis A Chemical and Statistical Study Copenhagen, E Munksgaard 1941)
Inflammatory tissues			
Acute	Increased	Metachromasia Heparin? (Sylvén, B Acta chir Scandinav [supp] 66 1, 1941)	Xanthomas in healing herpes zoster lesions
Chronic	Normal	Metachromasia Heparin? (Sylvén—cited above)	
Chronic parametritis	Normal	Metachromasia (Sylvén—cited above)	Frequently considerable amounts of foam cells ⁵
Cholesterol pleurisy	Normal		Large amounts of cholesterol in chronic pleural effusions
Syphilitic aortitis	Normal		The aortas with the highest cholesterol content (Björnsson—cited above)
Hand Schüller Christian disease	Normal and increased	?	Not known whether metachromasia is found in this tissue, but highly probable
Experimental Lesions			
Atheromatosis	Increased	All experimentally produced forms of atheromatosis are accompanied by reparative changes. In several forms metachromasia has been demonstrated (Erb ¹² , Ssolowjew ⁸)	
Arcus senilis in rabbits	Increased	Metachromasia	

presence of the tissue factor and that these carbohydrates themselves may be the tissue factor

It is, however, known that polyuronides of this type can be isolated from tissues which do not show ethanol-resistant metachromasia. In these tissues, there is reason to believe, the polyuronides are bound

more firmly to the proteins, as shown by the fact that their extraction free of protein is more difficult. This more firm binding may also inhibit the effect of the polyuronides on the deposition of cholesterol. This will, for instance, be the case of cutaneous tissue, which contains chondroitin-sulfuric acid³ but which does not give ethanol-stable metachromasia.

As mentioned, the ethanol-stable metachromasia can be a normal finding in an organ. In this case a deposition of cholesterol will be present in all human beings above a certain age limit, as has been shown in cartilage⁴ and cornea⁵.

When the metachromasia occurs in pathologic tissues, the deposition of cholesterol does not occur with normal serum cholesterol levels until the process has existed for some time (cholesterol in chronic inflammatory tissue⁶). When serum cholesterol is elevated, the deposition is more easily produced, and minor lesions will suffice to give rise to it (as seen in xanthoma in acute repair of tissue⁶ and experimentally in tendons⁷). In this group it will be natural to include the Schuller-Christian granuloma in which the cholesterol content is high though the serum cholesterol is mostly normal.

Finally, the metachromasia may increase in organs in which it already occurs, and thereby accelerate the deposition of cholesterol. Such a phenomenon can be expected in the intima and the media of the aorta as a result of mechanical injury^{8a} and in the granulation tissue of syphilitic mesaortitis.

Metachromatic tissue is always present in the human aorta and is described as due to the presence of chondroitin-sulfuric acid⁹. It is located in the intima and the luminal part of the media, places where cholesterol is found in the atheromatous vessels¹⁰. The metachromasia and the sulfuric acid that are present in the polyuronides are mainly found in the upper two thirds of the vessel¹¹. This is the typical localization of the cholesterol deposits in the hypercholesteremic type of

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4 Burger, M., and Schlomka, G. *Ztschr f d ges exper Med* **55** 287, 1925

5 Martineau, J. *Les xanthomes vrais et les pseudoxanthomes infectieux*, Thesis, Paris, Le Francois, 1927

6 Faber M. *Acta med Scandinav* **118** 436, 1944

7 Kusnetzowsky, N. *Virchows Arch f path Anat* **263** 205, 1927

8 Ssolowjew, A. (a) *Virchows Arch f path Anat* **283** 213, 1932, (b) **261** 253, 1926

9 Levene, P. A., and Lopez-Suarez, J. *J Biol Chem* **36** 105, 1918

10 Ssolowjew, A. *Virchows Arch f path Anat* **241** 1, 1923, **250** 259, 1924. Schultz, A. *Zentralbl f allg Path u path Anat* **239** 415, 1922

11 Jorpes, E., Holmgren, H., and Wilander, O. *Ztschr f mikr-anat Forsch* **42** 279, 1937

atheromatosis as seen in xanthomatosis and untreated myxedema. Thus the anatomic findings do not exclude the hypothesis.

When cholesterol is fed to rabbits in sufficiently small amounts, the normal aorta will take up practically none of this cholesterol. After certain experimental procedures, however, an uptake can be shown to occur. The main feature of the type of injury that will deposit cholesterol in the aorta under these conditions is the subsequent reparative processes, and these should show an increase of metachromasia.

This increase has been demonstrated in epinephrine sclerosis by Erb¹². Moreover, Ssolowjew^{8b} showed that cauterizing the aorta from without produced a metachromatic zone. This zone was demonstrable for two to three months. In later experiments¹³ he showed that the cauterization gave rise to increased deposition of cholesterol at the feeding of suboptimal doses of cholesterol only as long as the regenerative processes were active. No deposition could be produced coincident with the time when the metachromasia disappeared. Finally it may be mentioned that it is possible to produce aortitis experimentally with repeated injections of heterologous protein, probably accompanied by an increase of the metachromasia of the inflammatory tissue in the vessel. In this case suboptimal doses of cholesterol will result in deposition in the vessels, especially in the coronary vessels¹⁴.

In order to study this mechanism, an investigation was undertaken on the human aorta. As a measure of the carbohydrate-sulfuric acid ester content of the intima and media, the sulfate content of these tissues has been determined, on the assumption that all the sulfate is derived from these esters.

Fifty aortas have been examined. They came from autopsies at the Copenhagen County Hospital, Kommunehospitalet Copenhagen and the Medicolegal Institute of the University of Copenhagen. In all instances the aorta was prepared as soon as possible after the autopsy by removing the adventitia from the media and intima. These tissues were dried and hydrolyzed with fifth-normal hydrochloric acid for ten hours. The hydrolysates were extracted four times with ether, from the collected extracts cholesterol was determined by a quantitative Lieberman-Burchard reaction after alkaline hydrolysis.

The hydrolysates were filtered and evaporated to about 20 cc, and the sulfate was precipitated with barium chloride. When the sediment had settled, most of the supernatant was sucked off, and the sediment was transferred to a filter crucible, dried, ignited and weighed.

12 Erb, W. *Arch f exper Path u Pharmakol* **53** 173, 1905

13 Ssolowjew, A. *Ztschr f d ges exper Med* **69** 94, 1930

14 Schmitt. *Virchows Arch f path Anat* **296** 603, 1936. Brochs, H. *Experimentelle undersøgelser over lipoidaflejringer i coronararterierne hos kanner*, Thesis, Copenhagen, 1945.

The results are recorded in table 2, in which all the aortas are listed according to age in the four main groups normal, hypertension,

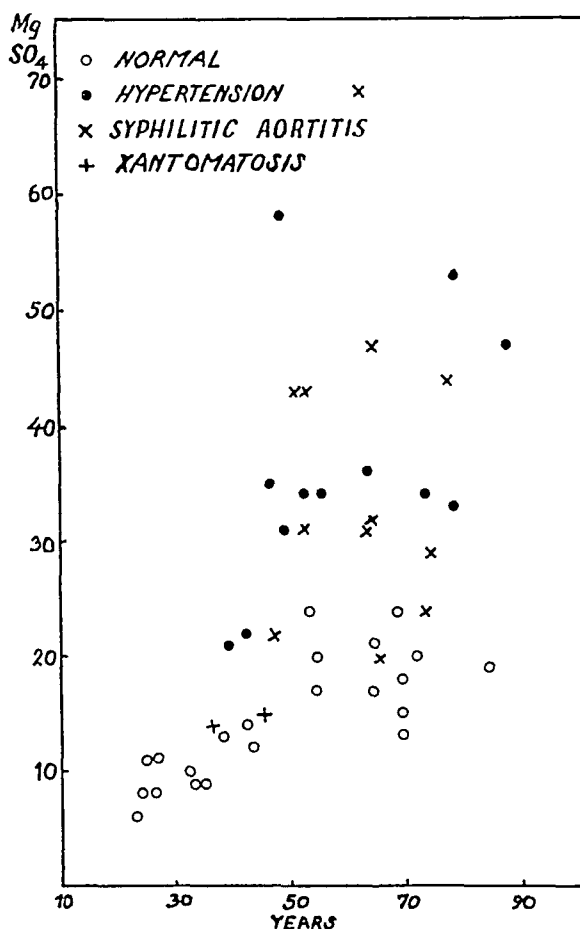
TABLE 2—*Aortas Examined*

No	Age	Sex	Weight of Dry Aorta, Gm	Choles- terol in Aorta, Mg	Sulfate in Dry Aorta		Blood Pressure	Diagnosis	Serum Choles- terol, Mg per 100 Cc
					Mg	%			
Normal									
1	23	M	4	31	6	0.17	150/70	Rheumatic fever	
2	24	M	5.5	26	8	0.24		Bullet wound	
3	25	M	5.9	24	11	0.26			
4	26	M	5.2	37	11	0.21			
5	26	F	5.6	34	8	0.23			
6	32	M	3.2	77	10	0.30			
7	33	F	3.2	25	9	0.28		Poisoning	
8	34	F	4.1	125	9	0.23	140/75	Chronic hepatitis	
9	38	M	4.6	63	10	0.34		Bullet wound	
10	42	F	4.8	70	14	0.29			
11	43	M	4.1	58	12	0.30	145/95	Poisoning	
12	53	M	7.5	197	24	0.31	130/		
13	54	M	7.8	115	20	0.21	145/80	Chronic hepatitis	
14	54	M	6.0	150	17	0.28	125/75	Duodenal ulcer	
15	64	F	9.4	320	17	0.18		Chronic hepatitis	
16	64	F	13.2	275	21	0.16	140/80		
17	68	M	9.5	390	24	0.26		Poisoning	
18	69	F	10.8	490	15	0.14	140/80	Chronic hepatitis	
19	69	M	11.1	355	13	0.12	120/70		
20	69	M	8.7	368	18	0.21	150/85	Cerebral hemorrhage	
21	71	F	13.5	572	20	0.15	160/90	Chronic hepatitis	
22	84	F	16.4	1,050	19	0.12	140/90	Heart disease	
Hypertension									
23	59	M	6.9	125	21	0.30		Coronary occlusion	
24	42	M	6.9	145	22	0.32			
25	46	F	8.6	250	35	0.40	200/110	Phlebitis	
26	48	M	15.7	815	58	0.37	230/120	Heart disease	
27	49	M	9.3	387	31	0.33		Coronary occlusion	
28	52	F	10.3	335	34	0.32	210/130	Pulmonary edema	
29	55	M	10.9	283	34	0.31	250/140	Hypertension	
30	65	F	16.8	1,160	36	0.22	190/110		
31	73	F	16.6	1,285	34	0.21	220/100	Chronic hepatitis	
32	78	F	32.8	1,680	53	0.16	210/120	Hypertension	
33	78	F	25.8	1,375	33	0.13	230/140	Cerebral hemorrhage	
34	87	F	22.0	2,310	46	0.16	230/150	Arteriosclerosis	
Syphilitic Aortitis									
35	47	M	8.3	325	22	0.27		Syphilitic aortitis	
36	51	M	28.0	356	43	0.15			
37	52	M	16.2	690	43	0.27	210/120		
38	57	M	16.2	670	31	0.18			
39	62	M	17.9	815	69	0.38	160/80		
40	6	M	19.0	706	31	0.16	165/100		
41	64	F	14.5	1,318	32	0.22	235/115		
42	64	M	19.0	860	47	0.25	205/85		
43	65	M	15.1	686	20	0.13			
44	73	M	12.9	1,410	24	0.19	190/75		
45	74	F	24.6	1,278	29	0.12	210/90		
46	77	F	10.9	1,210	44	0.40	180/60		
Xanthomatosis									
47	36	M	4.4	253	14	0.31		Xanthomatosis	400
48	45	M	7.5	430	15	0.20			398
Other Conditions									
49	17	M	3.7	19	15	0.42		Poisoning	
								Congestive heart disease	
50	17	F	2.7	33	9	0.33		Peripheral vascular calcifications	

syphilitic aortitis and xanthomatosis. Two aortas fall outside this grouping. One is that of a young girl with universal peripheral vascular calcifications, the other, that of a young man with congenital heart

disease. The latter had a relatively high sulfate content, while the first was normal.

The chart shows the relation between the total sulfate and age. It will be seen that in normal states the sulfate content of the aorta increases with advancing age, reaching a maximum at the age of 60, after which it remains constant. In hypertension there is shown a similar rise with age, but nearly all values lie above those for the normal group.



The sulfate content of the aorta in relation to age

in the same age group, and frequently above the highest normal values. The same is found in regard to syphilitic aortitis, though here the values are more scattered, with some relatively low values. Thus an increased amount of sulfate is found in these two forms of aortic lesions in which the deposition of cholesterol must be regarded as being accelerated on account of an increase in the tissue factor. Conditions are different in the 2 xanthomatous aortas. Each showed an entirely normal sulfate content. This was to be expected if it was the increase in the available cholesterol that determined the rate of deposition.

A somewhat different picture is seen when one is considering percentages. In the normal group there is a slight increase up to the age of 40, followed by constant values until the age of 60, when a distinct fall occurs. In the hypertensive group, compared with the normal group, the percentage is higher and there is the same tendency toward lower values in old age, though hardly as pronounced as in the normal group. No regularity is to be found in the aortas of persons who had syphilitic aortitis, nor is that to be expected, since the activity of the syphilitic processes must determine the sulfate content. Some of the aortas studied must undoubtedly be regarded as healed, and the predominant chemical residual lesion is a strong calcification. This applies especially to nos 40 and 43.

TABLE 3—*The Sulfate Content of the Aorta Corrected for Calcium and Cholesterol*

No	Weight of Dry Aorta, Gm	Choles terol in Aorta, Mg	Calcium in Aorta, Mg	Corrected Weight of Aorta, Gm	Sulfate in Dry Aorta		
					Mg	Per Cent of Total Aorta	Per Cent of Aorta Corrected for Choles terol and Calcium
Normal							
8	4.1	125	31	3.8	9	0.23	0.24
20	8.7	363	106	8.0	18	0.21	0.24
Hypertension							
27	9.3	387	99	8.7	31	0.33	0.36
33	25.8	1,375	3,410	15.5	33	0.13	0.25
34	22.0	2,310	2,112	14.2	46	0.16	0.32

The rise observed in the sulfate content with advancing age will thus be due mainly to the increasing weight of the organ. There are several reasons for this increase of weight. Among these are the calcium and cholesterol deposits which do not participate in the growth and metabolism of the vessel. With the assumption that the cholesterol measured represents the total lipid and that the calcium was deposited as three molecules of calcium phosphate to one molecule of calcium carbonate ($3 \text{ Ca}_3 (\text{PO}_4)_2, \text{CaCO}_3$), the "metabolic active" tissue can be calculated. This correction is at any rate not too high in view of the fact that actually one finds considerable amounts of lipids besides the cholesterol. Applying the correction as seen in table 3, one sees that the percentage content of sulfate is at least at the same level in the senile vessels as in the younger vessels. The active part of the vessel thus appears to contain a rising percentage of sulfate during the whole age interval investigated.

COMMENT

In the foregoing pages an attempt has been made to establish a common rule for a series of extracellular cholesterol depositions and to confirm this rule by the study of a single organ. The human aorta gives in the main confirmation of the theory advanced. As mentioned, sulfuric acid esters are found in places corresponding to the cholesterol deposits, and the increasing amount of cholesterol with rising age corresponds to a rising amount of sulfate. In cases of hypertension one finds by absolute measurement and by percentage an increase in sulfate corresponding to the increased rate of deposition of cholesterol. The same is more or less true of the majority of cases of syphilitic aortitis, but here the activity of the inflammation seems to determine the sulfate content. One might perhaps have expected a somewhat higher sulfate content in old age, considering the distinctly increasing rate of cholesterol deposition with age.

There remains a discussion of why the cholesterol is deposited more easily in the sulfuric acid ester-containing tissue than in other tissues.

It is generally accepted that the cholesterol is not synthesized at the place of deposition but is brought into the tissue from the plasma. The cholesterol which enters the tissues will thus be in the form of the serum lipoproteins. The question is: How will the lipoproteins react with the sulfuric acid esters to give rise to cholesterol precipitation? The investigations of Chargaff¹⁵ may suggest an answer. When heparin is allowed to act on lipoproteins, the heparin occasionally enters the lipoprotein. In the majority of cases, however, there occurs a liberation of the lipid, the heparin assuming the place of the lipid in the protein. The latter mechanism seems to fit the conditions found in the wall of the aorta and in other places where cholesterol is deposited extracellularly. It must, however, be mentioned that the serum lipoproteins will not release their lipids on being treated with heparin in their native state.

One phenomenon deserves further mention. The absolute sulfuric acid content of the aorta increases with age, but the percentage content hardly shows the same rise. Accordingly, one would expect that the rate at which the cholesterol is deposited would rise at a rate that would be not much higher than the rate of the increase in weight. This is, however, not the case. The weight of the dried intima and media of the aorta will rise following the equation $\log \text{ weight} = 0.3171 + 0.0100 \times \text{age}$,¹⁶ while the cholesterol content follows the equation $\log \text{ cholesterol} = 0.9792 + 0.0241 \times \text{age}$. This means

15 (a) Chargaff, E., Ziff, M., and Cohen, S. S. *J. Biol. Chem.* **136** 257, 1940. (b) Chargaff, E., *ibid.* **142** 491, 1942.

16 Faber, M., and Lund, F. To be published.

that the weight of the vessel increases only about five times during life, while the rate of the cholesterol deposition during the same time rises ten to twenty fold

It is possible that this increased rate of deposition is due to changes in the lipoproteins of the serum¹⁷ Reference is here made to a frequently mentioned but still rather vague phenomenon, the cholesterolytic property of serum. Several authors have indicated that while cholesterol when added to serum from younger persons increases the amount of cholesterol dissolved probably as lipoprotein, the same is rarely true in regard to older persons, in whose case one may even observe a precipitation of the cholesterol already dissolved in the serum. This negative cholesterolytic property should be most pronounced in cases of hypertension. The phenomenon, however, requires further study. Since ample amounts of free cholesterol may be found in vessels with a high cholesterol content—even in crystalline form—it may be that a negative cholesterolytic property of the serum accelerates the deposition of cholesterol.

SUMMARY

On the basis of the literature it is shown that the organism contains a number of extracellular cholesterol deposits and that these are always to be found in tissues which beforehand contain substances that can be stained metachromatically. It is therefore possible that these substances are responsible for the depositions of cholesterol.

In the human aorta one finds an increasing content of sulfate as a measure of the metachromatically stainable carbohydrate-sulfuric acid esters with advancing age. Higher values are found in cases of hypertension and in most cases of syphilitic aortitis, corresponding to the increased cholesterol contents. The sulfate content of the xanthomatous aorta is normal.

The paper discusses the details of the mechanism involved in the deposition of cholesterol in a tissue containing sulfuric acid esters of the kind mentioned.

¹⁷ Alvarez and Neuschloss. *Klin. Wchnschr.* **10** 244, 1931. Eck, and Desbordes. *Compt. rend. Soc. de biol.* **118** 498, 1935. Obrecht. *Ueber das cholesterolytische Vermögen des Blutserums im Alter und bei Hypertension*, Thesis, Bern, 1941.

THE HUMAN AORTA

Influence of Obesity on the Development of Arteriosclerosis in the Human Aorta

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AND

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OBESITY is quite often the first visible link in the chain of events which after some years will give rise to signs of arteriosclerotic disease

It is a question how great an influence the obesity in itself will have on this development—whether the sclerosis is a direct result of the obesity and the biochemical and other changes that follow this condition, or whether the obesity affects the vessels through a more complicated mechanism. A few workers have tried to study this problem

In a study of 1,250 aortas Wilens¹ correlated the weight of the patient with the degree of arteriosclerosis. The sclerosis was evaluated by macroscopic appraisal at the autopsy, taking into account not only the findings in the aorta but in most cases the state of the other vessels also. He reported that as compared with the degree of sclerosis found in patients of normal weight the sclerosis observed in the obese was greater and that in the emaciated less. In a later work² he extended these studies and showed that premortal emaciation is followed by a decrease of the sclerosis of the aorta. Similar results were obtained by Eskola³ using a similar technic. The value of these studies is somewhat diminished, however, by the great difficulty encountered when a quantitative evaluation of the findings has to be made on the basis of the macroscopic appearance of the vessel.

A more exact evaluation of the sclerosis should be possible if a quantitative study is made of any of the substances of the wall of a vessel, especially if these substances can be shown to bear a well defined relation to the sclerotic changes. On the basis of determinations

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1 Wilens, S. L. *Arch Int Med* **79** 129, 1947

2 Wilens, S. L. *Am J Path* **23** 793, 1947

3 Eskola. *Duodecim* **64** 443, 1948

of cholesterol and calcium Rosenthal⁴ was not able to find any interdependence between obesity and the arteriosclerotic changes Björnsson,⁵ who used a somewhat similar technic, noted a slight but not significant increase of the sclerotic changes in obesity

It is generally accepted that obesity in many cases will be complicated by diseases which in themselves may evoke an increased or rather an acceleration of sclerosis The most important of these diseases is hypertension The evidence that increased blood pressure will produce sclerotic changes of both the central and the peripheral arteries and of the veins has recently been summarized by Moschowitz⁶ The question still remains whether obesity is able to increase the sclerosis when hypertension is present In Wilens' series the effect of weight was evident also in the hypertensive group

Diabetes mellitus is another complication of obesity which may influence the rate of sclerosis The mechanism of the diabetic sclerosis is not known, probably the causes are complex In the sclerosis seen late in diabetes, hypercholesteremia must, however, be taken into account, even if it has been of short duration

Hypercholesteremia will probably also be the determining factor for the sclerosis seen in Cushing's disease and in myxedema The same will be the case in hereditary hypercholesteremia and in xanthomatosis, in which a relative increase in the cholesterol content of the aorta is a typical finding (Faber⁷) In a series of 80 soldiers dying from coronary occlusion French and Dock⁸ observed some obesity in 73 The description given of the aortas is however so close to the type found in hypercholesteremia that these aortas cannot be accepted as showing a direct effect of obesity on vascular sclerosis so long as no serum cholesterol determination is reported .

The moderate degree of hypercholesteremia reported by Gildea, Kahn and Man⁹ and later by Kornerup¹⁰ in persons of pyknic type must, however, be considered These persons will presumably for the most part belong to the obese group, and this will influence the degree of sclerosis seen in cases of obesity

4 Rosenthal, S R Arch Path **18** 473 and 660, 1934

5 Björnsson, J Arteriosclerosis A Chemical and Statistical Study, Copenhagen, 1941

6 Moschowitz, E Vascular Sclerosis, New York, Oxford University Press, 1942

7 Faber, M Acta med Scandinav **125** 210, 1946

8 French, A J, and Dock, N J A M A **124** 1233, 1944

9 Gildea, E F, Kahn, E, and Man, E B Am J Psychiat **92** 1247, 1936

10 Kornerup, V Familiær hypercholesterolaemi og xanthomatose, Thesis, Copenhagen, 1948

In comparing the cholesterol content of the aorta with the serum cholesterol it has not been possible to show any correlation so long as the serum cholesterol remained inside the normal range. With serum cholesterol above the normal levels, however, such a relation could be found.⁷ Studies on the serum cholesterol of patients with clinical signs of coronary vascular disease, angina pectoris and coronary occlusion indicated, however, that the lower the serum cholesterol the higher the age at which the coronary sclerosis became manifest, a finding now substantiated by Morrison and co-workers.¹¹ These results indicate that changes of serum cholesterol level inside the normal ranges perhaps during a longer period can be of importance for the rate at which cholesterol is deposited in the wall of the vessel.

Of great interest is the question whether a diet low in cholesterol will decrease the concentration of cholesterol in the serum. Studies on the rice diet show that this can be the case.¹² However, other diets not low in cholesterol but low in calories seem to give the same result. The low incidence of sclerosis found in the Chinese by Oppenheimer¹³ and later by Snapper¹⁴ could be explained by these facts. Weiss and Minot¹⁵ claimed that patients dying of pulmonary tuberculosis show less sclerosis than was to be expected. This would follow from the poor nutritional state, perhaps complicated by the persistent hypotension seen in chronic infectious diseases.

We have found the problem of the relationship of obesity and arteriosclerosis still undecided and have therefore, in a series of cases, tried to get as exact figures for the relationship as possible.

To grade a sclerotic vessel macroscopically post mortem, even when the age of the subject is known, is extremely difficult, and there can be no doubt that each arterial system must be considered by itself. A parallel change in the different parts of the arterial system cannot be expected. This is shown in the preponderance of males among patients with coronary occlusion in the younger age groups and in the earlier and more marked calcification seen by roentgenogram in the abdominal aorta in women.¹⁶

We have restricted this study to the intima and media of the total aorta, and as a basis for the evaluation we have studied three factors—the dry weight, the cholesterol content and the calcium content.

11 Morrison, L. M., Hall, L., and Chaney, A. L. *Am J M Sc* **216** 32, 1948.

12 Kempner, W. *Bull New York Acad Med* **22** 358, 1946.

13 Oppenheimer, F. *Chinese M J* **39** 1067, 1925.

14 Snapper, I. *Chinese Lessons to Western Medicine*, New York, Interscience Publishers, Inc., 1941.

15 Weiss, S., and Minot, S. R. *Nutrition in Relation to Arteriosclerosis*, in Cowdry, E. V. *Arteriosclerosis*, New York, The Macmillan Company, 1933, pp 233-248.

16 Petersen, G. F. *Acta radiol (supp)* **39** 1, 1941.

MATERIAL

The aortas studied were collected at autopsies in the hospitals of Copenhagen and in the Medicolegal Institute of the University of Copenhagen. All the studied cases in which sufficient data were available have been used except the following groups: all cases of syphilitic aortitis and cases in which valvular heart diseases was present, cases in which hypercholesteremia or diabetes mellitus was present, and cases of Cushing's syndrome or myxedema. Cases described in previous publications have been used when sufficient data were available. The majority of the cases of normal status were collected at the Medicolegal Institute and consisted primarily of cases in which sudden death occurred as a result of an accident. The rest of the material has been collected so as to get the greatest number of cases with hypertension and obesity. Most of the hospital patients died less than forty-eight hours after admission. Of the rest, only a few had wasting diseases. We therefore think this material will not be affected by Wilens' ² finding that premortal emaciation should decrease the sclerosis. The diet, especially the lipid content of the Danish diet, has not been changed during the four years these cases have been accumulated to a degree that will make them incomparable.

PROCEDURE

The total aorta from 1 cm above the aortic valve to the bifurcation was prepared free of adventitia soon after the autopsy. The error introduced by a slightly inaccurate dissection will turn up only in the weight: the greatest part of the cholesterol and the calcium being in the luminal part of the media and in the intima. The tissue was cut up with scissors and dried in a vacuum over sulfuric acid for twenty-four hours, and the weight determined. This weight has been used throughout, although it is not the correct dry weight of the tissue, but somewhat higher.

The dry tissue or an aliquot was hydrolyzed for three hours in fifth-normal sodium hydroxide and extracted four times with ether, and the cholesterol was determined from the collected ether extracts by a quantitative Lieberman-Burchard reaction.

The calcium was determined in an acid hydrolysate of the tissue by precipitation with oxalate at pH 5.8 and permanganate titration.

In most of the cases the weight and the height were obtained in the hospital or at autopsy. Only in a few cases did we have to rely on the simple evaluation, normal, obese and very obese. The normal weight of each patient was calculated according to the tables given by Fisk and Crawford,¹⁷ this calculation taking into account the height and the sex of the patient. No correction was made for the fact that the figures of Fisk and Crawford were obtained on fully dressed persons, because the lack of shoes probably would compensate for the lowering of the weight due to lack of clothes. Obesity has in this study been defined as a weight more than 10 per cent above the calculated normal weight. A group of very obese persons with weights more than 25 per cent above the normal will be considered separately.

The highest blood pressure measured during the terminal hospitalization has been used. In some cases earlier measurements were available and have been of use in cases in which the premortal blood pressure represented the blood pressure

¹⁷ Fisk, E. L., and Crawford, J. R. *How to Make the Periodic Health Examination*. New York: The Macmillan Company, 1927.

during shock. Blood pressures above 160 mm of mercury systolic and 90 mm diastolic have been registered as hypertension.

In a relatively large group no data on blood pressure were available, especially in the cases of sudden death. In these, however, the heart weight was used. We have classified such cases as cases of hypertension when the heart weight was more than 2.5 times the standard deviation, that is, more than 100 Gm higher than the normal weight when sex and height were taken into consideration.¹⁸ In the cases in which the heart weight and the blood pressure were known, an elevated blood pressure was considered more significant than the heart weight. This discrepancy was seen in only few cases, however.

TABLE 1—*Distribution of Material (408 Aortas) According to Sex, Blood Pressure and Body Weight*

Sex	Normal Blood Pressure		Hypertension		Total
	Normal Weight	Obesity	Normal Weight	Obesity	
Male	141	22	39	38	240
Female	77	19	38	34	168
Total	218	41	77	72	408

TABLE 2—*Distribution of Material (408 Aortas) According to Age, Blood Pressure and Body Weight*

Age	Normal Blood Pressure		Hypertension		Total
	Normal Weight	Obesity	Normal Weight	Obesity	
Below 20	10	0	0	0	10
20-29	32	0	1	0	33
30-39	47	10	7	1	65
40-49	28	11	12	12	63
50-59	38	9	10	16	73
60-69	39	9	18	21	87
70-79	17	1	20	18	56
80-89	6	1	6	4	17
Above 90	1	0	3	0	4
Total	218	41	77	72	408

A total of 408 aortas were used in this study. The distribution in body weight and blood pressure groups according to sex is shown in table 1. The distribution according to age is shown in table 2.

The great increase in the degree of sclerosis with increasing age makes it necessary in some way to eliminate the age factor from the evaluation of the sclerosis. It has been shown by Björnsson⁵ that the logarithm of the cholesterol and calcium content of the vessel wall rises rectilinearly with age, and the same is found for the dry weight of the

tissue To eliminate the effect of age, we have calculated the formula for this line for the three factors studied, and the evaluation of the sclerosis will be on the basis of the deviations from this line as found in each aorta

These deviations have been used for the construction of distribution curves, and the distribution curves of the different groups of cases will be compared

WEIGHT

The dry weight of the normal intima and media of the total aorta is found to vary between 2 and 26.5 Gm. On the basis of the normal material the formula for the rise in weight with age when the weight is measured in grams is found to be $\log \text{ weight} = 0.3171 + 0.100 \times \text{age}$

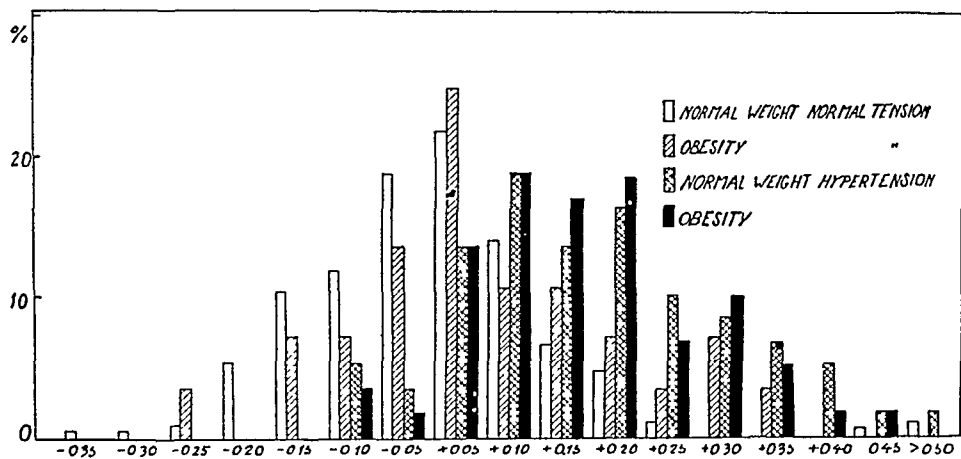


Chart 1—The distribution curves for the dry weight of the intima and media of the aorta around the calculated normal line in the four groups studied

In chart 1 is seen the distribution curve for the weight in the four groups under study when the group with normal weight and normal blood pressure is used for the calculation of the standard line. The spread around the mean is fairly large, but the cases with obesity and normal blood pressure show a distribution curve of exactly the same mean and spread as is found for the group with normal weight. In the two groups with hypertension the results are similar. Both show a displacement toward higher values, but the displacement is the same in both groups with the result that the two curves are practically identical.

These results show that hypertension will produce a rise in the weight of the wall of the aorta to values higher than was to be expected at the age of death. It is, however, not possible to show any effect specific for the obesity when the material is broken up according to blood pressure.

CHOLESTEROL

The cholesterol content of the aorta was found to vary between wider limits than the weight of the tissue. The lowest cholesterol content found was 11 mg and the highest 3,130 mg. By means of the normal material the formula for the cholesterol content of the vessel measured in milligrams is found to be $\log \text{cholesterol} = 0.9792 + 0.0241 \times \text{age}$. The distribution curves for the four groups are found in chart 2. As was the case with the dry weight, the distribution curves for the cholesterol in the groups of normal weight and obesity show coincidence both in maximum and in spread in the group with normal blood pressure. In this regard also the cases of hypertension show a displacement toward higher values as was to be expected with the normal weight and the obesity groups behaving identically.

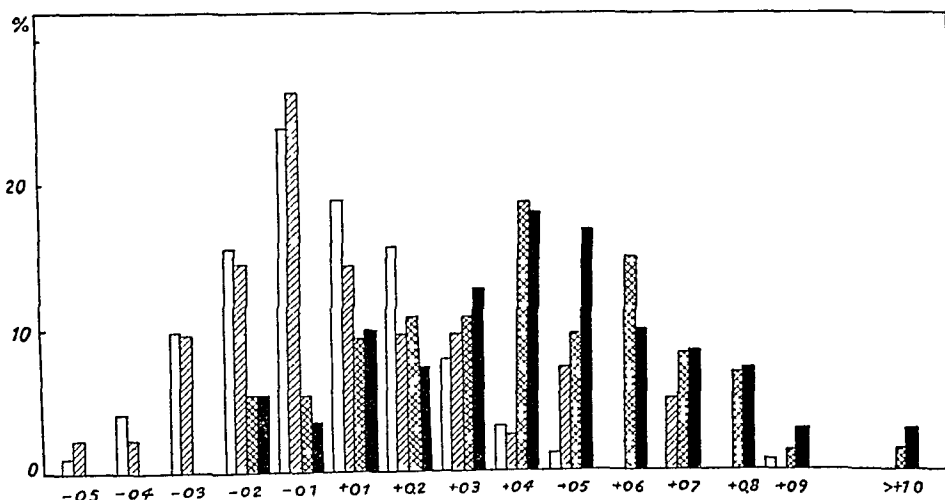


Chart 2—The distribution curves for the total cholesterol content of the intima and media of the aorta around the calculated normal line for the four groups studied. See chart 1 for key.

The result of the study of the cholesterol content of the aorta thus followed that of the dry weight in not showing any effect of obesity as such.

CALCIUM

As was to be expected, the spreading of the calcium values was still greater than was that of the cholesterol values. The lowest value found was 4 mg and the highest 3,410 mg. The formula for the rise in calcium content in relation to age will therefore show a steeper rise than was found in the other curves. Expressed as milligrams, the formula is found to be $\log \text{Ca} = 0.3060 + 0.0318 \times \text{age}$. The deviations from this line are found in chart 3. The spreading of the values around the calculated line is somewhat greater than with the dry

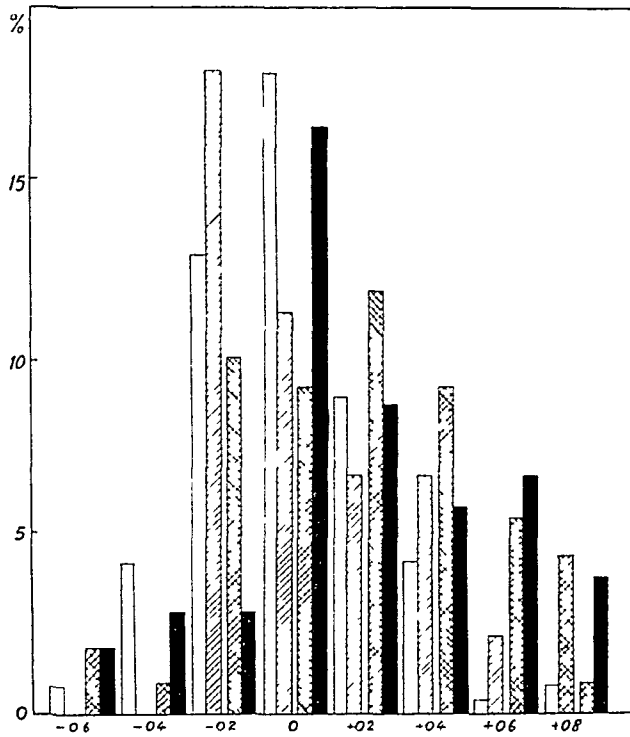


Chart 3—The distribution curves for the calcium content of the intima and media of the aorta around the calculated normal line for the four groups studied. See chart 1 for key.

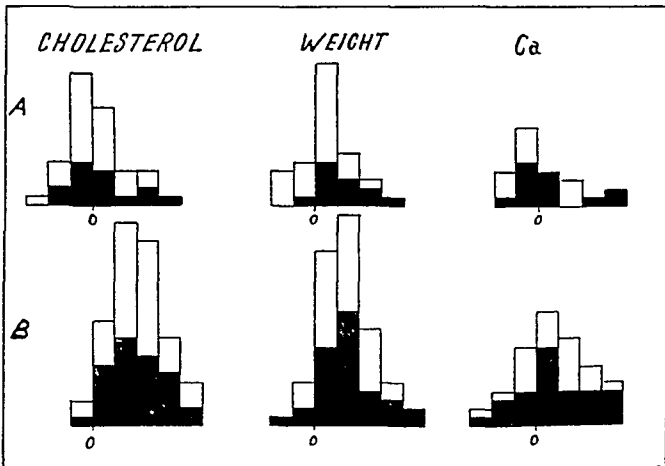


Chart 4—The distribution of the very obese (*o*) among all the obese in the distribution curves from charts 1 to 3 in two series. *A*, normal blood pressure, *B*, hypertension. Black represents weights more than 25 per cent above the normal, white weights between 10 and 25 per cent above normal.

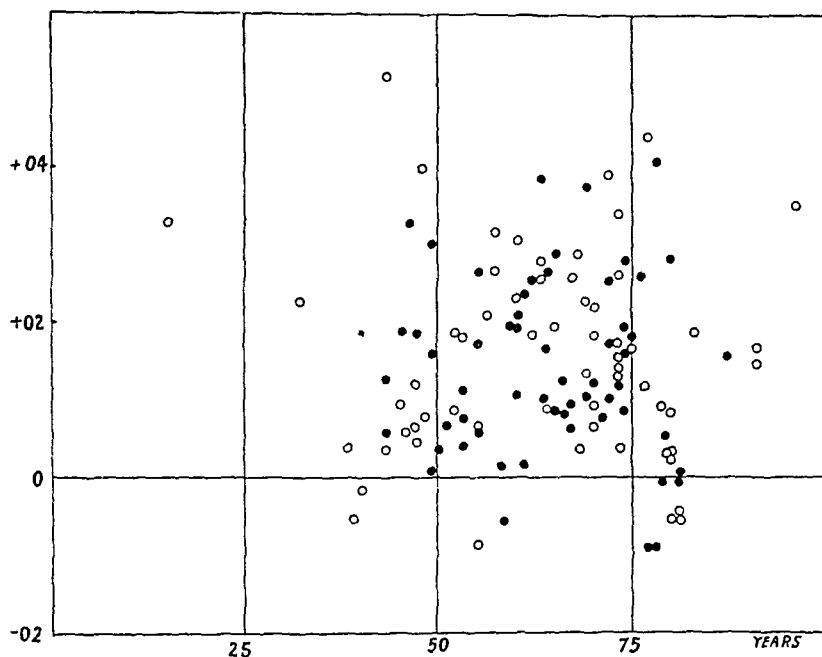


Chart 5—The deviations in log dry weight from the calculated normal line in relation to age. White circles represent deviations associated with normal body weight, black circles, deviations associated with obesity.

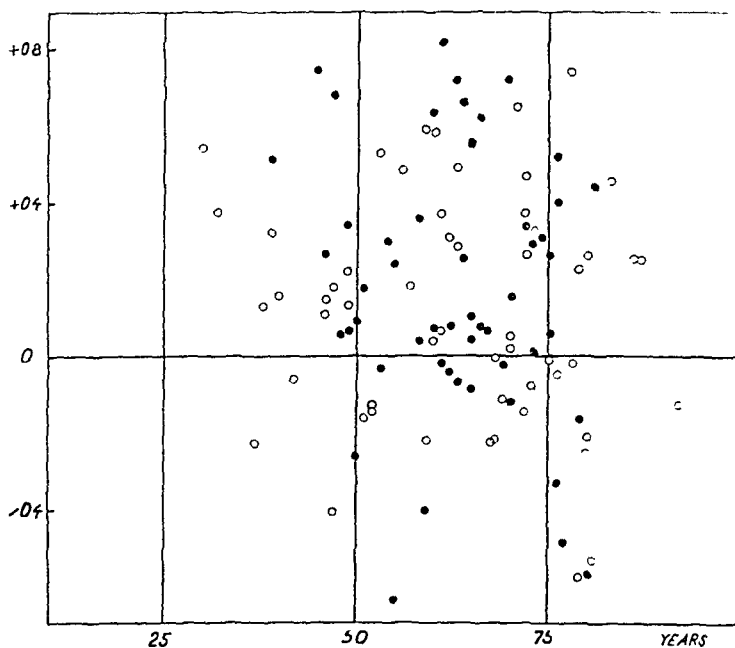


Chart 6—The deviations in log cholesterol from the calculated normal line for the hypertensives in relation to age. White circles represent deviations associated with normal body weights, black circles, deviations associated with obesity.

weight and the cholesterol. Remarkable is the relatively large number of normal and low normal values found in cases of hypertension. The relative positions of the distribution curves are, however, as found with the other factor studied, with no sign of any specific effect of obesity.

It might be claimed that the limits for obesity have been drawn too low in this study and that an effect could be masked in this way. To settle this point, the obese material has been divided into two groups, one with overweight between 10 and 25 per cent and the other with overweight more than 25 per cent. As seen from

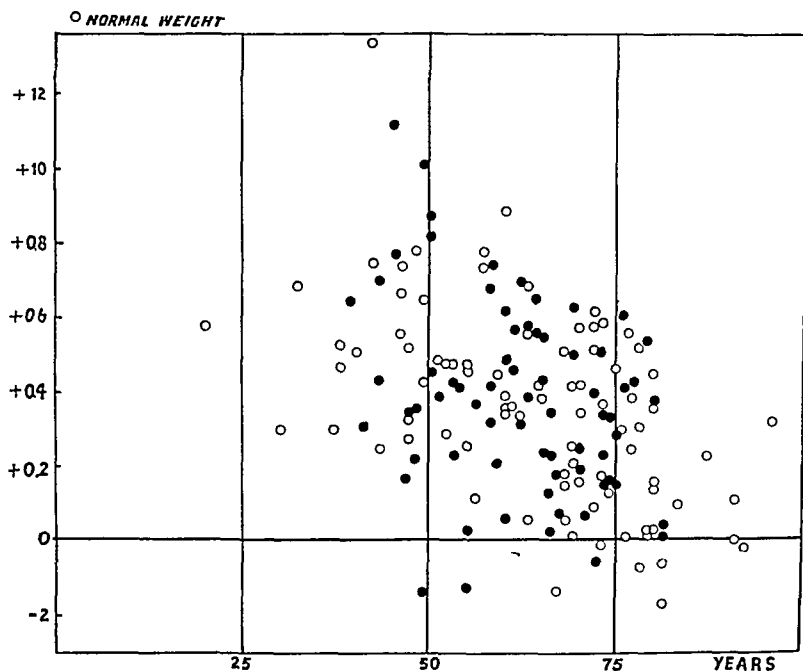


Chart 7—The deviations in log calcium from the calculated normal line for the hypertensives in relation to age. White circles represent deviations associated with normal body weights, black circles, deviations associated with obesity.

chart 4, this division does not invalidate the conclusions drawn, the very obese being evenly distributed in the obese material.

In this material it has not been possible to show any variations on account of sex or height. An analysis of the blood pressures measured did not show any increase in the degree of sclerosis depending on how much the blood pressure was elevated above the upper normal limits.

In the distribution curves for the cases of hypertension it was shown that some of the values found were fairly low, often below the normal mean. These low values may have a different significance than the higher values found in most of the cases of hypertension. One possibility is that this was an effect of age.

In chart 5 is seen the deviation from the calculated line in relation to age for the dry weights of the aortas of the hypertensive group. No relation to age can be seen. In regard to cholesterol, however, there can be shown a definite age dependence, as seen in chart 6. When the material is divided into age groups an interesting difference is found. Up to the age of 50 years even the cases with the least deviation are found far away from the calculated line. In the age group between 50 and 75 years, the largest deviations are missing, and the smallest approach the normal line. In the group above 75 years this movement toward the normal is continued, and when 80 years is passed a normal distribution is found.

The behavior of the calcium is seen in chart 7. The distribution of the values in the figure resembles mostly the distribution seen in regard to the weight. However, the number of normal, and especially of low normal, values seems to rise with increasing age.

This normalization of the values with increasing age can be explained only if it is assumed that the hypertension which is seen in these very old people is of another type than the one met with in the younger age groups. The elevation of blood pressure is moderate, in most cases below 200 mm systolic, and is presumably to be considered a result of the normal sclerosis of the vessels and not as a disease in itself.

SUMMARY

The influence of obesity on arteriosclerotic changes has been studied on the basis of determinations of aortic dry weight, cholesterol and calcium in 400 aortas.

The rise of these three factors with age has been calculated. It can be shown that hypertension gives a rise above what should be expected according to age.

Obesity itself, however, has no effect on any of the factors studied when the presence of hypertension is taken into account.

A PREINVASIVE CARCINOMA OF THE UTERINE TUBE

R R GREENE, M D
AND
G H GARDNER, M D
CHICAGO

PRI-MARY carcinoma of the uterine tube is a rare lesion. In all of the reported cases the lesion was relatively well advanced when discovered, except for a case reported by Mitchell and Mohler¹ in 1945. In their case the tumor was discovered during routine examination of small segments of tube excised for the purpose of sterilization. Subsequent total hysterectomy and bilateral salpingo-oophorectomy were performed. However, the Mitchell-Mohler specimen is a well developed small tumor, since it had almost obliterated the lumen of the tube, had extended into the muscle and had invaded lymph spaces and small blood vessels.

The carcinoma of the tube to be presented here was discovered accidentally. The essential pathologic process was pelvic endometriosis and total hysterectomy and unilateral salpingo-oophorectomy were performed. The tube and ovary were not included in the tissues sent to the hospital laboratory but were diverted to our special study of broad ligaments. During microscopic examination, attention was attracted to a particular area in the tube because of several mitotic figures in one microscopic field (mitotic figures are extremely rare in tubal epithelium). Further examination made it obvious that there was a minute preinvasive carcinoma of the uterine tube. The tumorous area occupied approximately a tenth of the total cross section area of the endosalpinx. There was histologic evidence of follicular salpingitis, with its characteristic fenestration of sealed plicae in the endosalpinx. The tumor had replaced the epithelium of three of these glandlike spaces and portions of several others. There was no invasion of the underlying musculature, and no invasion of blood vessels or lymph spaces. In fact, there was no evidence of invasion at all.

The epithelium was piled up, and in some areas truly stratified. Apparently the tumorous epithelium was progressing along the lumen of several of the glandlike spaces, and in each there was a point of abrupt transition from tumorous to normal epithelium. Mitotic figures

From the Departments of Obstetrics and Gynecology, Northwestern University Medical School and Wesley Memorial Hospital.

1 Mitchell, R M, and Mohler, R W. *Am J Obst & Gynec* 50:283, 1945

were frequent. Many of these mitotic figures were abnormal in appearance, tripolar figures being common. The nuclei in general were hyperchromatic, and the nucleoli were prominent. The nuclei varied in size and shape, and a few truly giant nuclei were present, also an occasional giant mitotic figure was observed.



Fig 1—*A*, low magnification of preinvasive carcinoma of the uterine tube, showing total extent of carcinomatous area (thicker and darker-staining epithelium) *B*, medium magnification, showing stratification and nuclear variations

Both the secretory and the ciliated types of cells had apparently participated in this carcinomatous process. There was however, some variability in the appearance of different areas. In some, the cytoplasm of the ciliated cells still stained lightly, and the nuclei were somewhat

vesicular and had the usual shape, however, they were approximately twice normal size. The secretory cells were markedly enlarged, crowded and hyperchromatic. Mitotic figures were noted in these same areas, but the type of cell which was undergoing mitosis could not be determined.

In other areas the cytoplasm of all cells stained more deeply. The nuclei of ciliated cells were elongated and frequently irregular in shape. The amount of chromatin seemed to be increased. In some areas cells of secretory origin and those of ciliated cell origin were not distinguishable except for the presence of cilia on the free or luminal border of the latter.

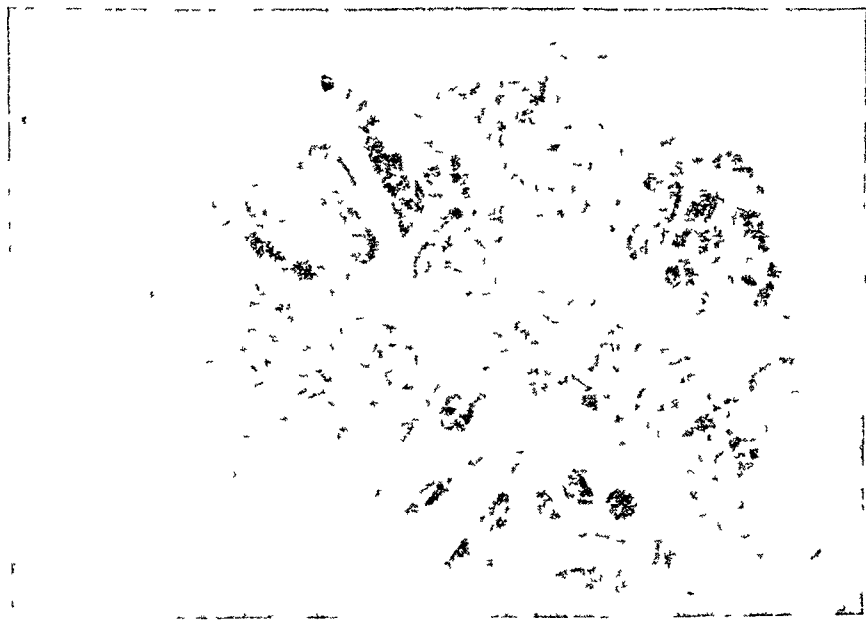


Fig 2—High magnification, showing giant mitotic figure

In still other areas dedifferentiation had taken place and there were no cilia. In some areas anaplasia was marked. The nuclei were small and relatively uniform in size, but the gross increase in the size of the nucleolus or nucleoli was particularly obvious. Elsewhere a small portion of the tissue showed definite nuclear pleomorphism, here the nuclei were hyperchromatic and varied markedly in size and in shape.

The noncancerous portion of the tubal epithelium was quite high, with the usually varying proportion of ciliated and secretory cells. In some areas the epithelium was pseudostratified. In a few areas there was a piling up of the cells to produce intraluminal "tufts" or small "papillae." There were a few large, atypical-appearing cells with bizarre-shaped, atypical nuclei. One mitotic figure was noted. These findings denote a moderate degree of endosalpingeal hyperplasia.

Subsequent to the discovery of this small tumorous area, multiple blocks were made from the remaining portion of the tube, and more sections were made from the original block. The tumor was found to extend for a very short distance. No evidence of tumor was found in any of the other blocks.

The discovery of this tumor was, of course, purely fortuitous. Since primary carcinoma of the fallopian tube is extremely rare, it is not expected that it will be found in such an early preinvasive stage in many cases.

Our patient is under observation, it is now eight months since the operation, she is well and free from objective evidence of recurrence, no further treatment is planned.

Laboratory Methods and Technical Notes

A PRACTICAL DEVICE FOR DEMONSTRATING AIR EMBOLISM

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THE QUANTITATIVE and qualitative demonstration of air or other gases that may be present in the cardiac ventricles, the pleural sacs or other cavities of the body must not be neglected in the course of forensic autopsies. Furthermore, it is a questionable practice to arrive at the diagnosis of air embolism, of pneumothorax and like conditions by retrospection, or, so to say, circumstantial evidence. The method which is recommended in the textbooks of pathology or the handbooks for autopsies,¹ i e., the opening of the cavity under water is rather primitive and not always practicable even when the proper precautions are taken.

In the many autopsies at the Coroner's Office in Cleveland (more than 1,500 autopsies in the last four years) the need for a simple apparatus for making such demonstrations compelled me to develop the one herein described. This device is constructed of such materials as are readily available in any laboratory and is so simple that its use should present little difficulty.

DESCRIPTION OF THE DEVICE

As shown in figure 1, the apparatus consists of the following parts:

A One wide mouth glass bottle (2 or 3 ounce [60 to 90 cc] capacity) fitted tightly with a two hole rubber stopper.

B Two sections of glass tubing of approximately 3 mm inside diameter, each bent at an angle of 120 degrees. One of these sections should be longer than the other. The shorter one should reach just through the stopper and be even with the inner surface of the stopper. The longer one should reach to within 1 or 1.5 cm of the bottom of the flask. Both tubes should fit tightly into the holes of the stopper.

C One separatory funnel (60 to 100 cc capacity, pear shaped) connected to the longer section of bent glass tubing by rubber tubing 100 cm in length (*F*). In my experience an amber, pure gum rubber tubing such as is used on blood diluting pipets has proved satisfactory.

D One transfusion needle, no 14 or 15 gage, 4 or 5 cm in length, connected to the shorter glass tube by a short section of rubber tubing not exceeding 5 cm in length (*F*).

From the Cuyahoga County Coroner's office

1 Anderson, W. A. D. Pathology, St. Louis, C. V. Mosby Co., 1948, pp 126 and 127. Boyd, W. A. Textbook of Pathology, ed 5, Philadelphia, Lea & Febiger, 1947, pp 83 and 476. Saphir, O. Autopsy Diagnosis and Technique, ed 2, New York, Paul B. Hoeber, Inc., 1946, pp 75 and 189. Moritz, A. R. Pathology of Trauma, Philadelphia, Lea & Febiger, 1942, p 132. Gradwohl, R. B. Clinical Laboratory Methods and Diagnosis, ed 4, St. Louis, C. V. Mosby Co., 1948, vol 2, pp 1855 and 1866.

E Two pinchcock clamps, one for each length of tubing. They may be of the spring type or of the household syringe type. The latter will prove advantageous if the gas collected is to be transported for analysis.

The entire system is filled with liquid petrolatum so that when the funnel is at a level with the upright bottle the oil fills only about one half of the funnel.

TECHNIC

In operation the funnel is first raised to a position 30 or 40 cm. above the level of the upright bottle (position one in fig. 2). All the cocks are opened and the position is retained until every trace of gas has been driven from the system through the needle which is thereby coated on the inside by a film of oil. After

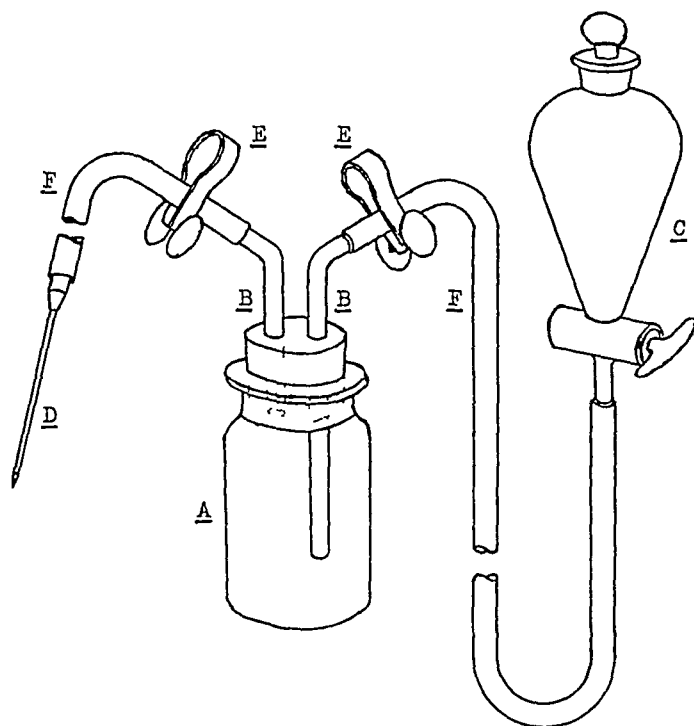


Fig. 1—Apparatus for the demonstration of air embolism. See text for the explanation of this diagram.

all air has been expelled, the cocks are closed and the funnel lowered once again to its original position.

As a precautionary measure and control, the airtightness of the whole system should be tested before operation. This is done by inserting the needle into musculature or skin and attempting aspiration in the manner described in the next paragraph.

To make the test, the bottle is inverted and the needle inserted in the cavity in question. When the needle is in position, all cocks are opened. The funnel is lowered about 70 to 90 cm., or until adequate suction is created. Thereby the contents of the cavity are aspirated. These may consist of air or other gases, either pure or mixed with blood or other liquid. Any gas or liquid entering this system may be observed through the wall of the short bent glass tubing. In case of a positive test, gas bubbles will collect in the bottle above the level of the oil. If desired, this gas may now be saved for further examination by closing all the cocks and returning the bottle to its upright position.

As a less satisfactory substitute for this apparatus, I have used a 30 cc glass syringe which was half-filled with water and fitted tightly to a no 15 gage needle. All air bubbles must have been removed and the whole thing checked for air-tightness. The test may then be made by inserting the needle as described in the foregoing paragraph and applying slow and careful suction. Here the principal difficulty lies in the inability to control the suction applied, so that under excessive negative pressure the gases normally dissolved in the blood may be liberated.

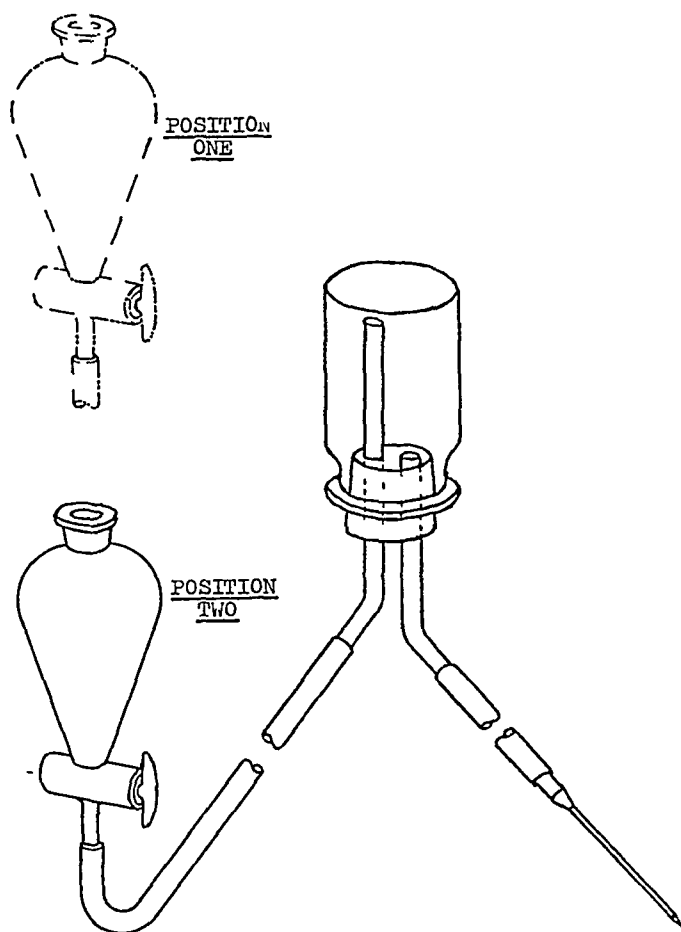


Fig 2—The two positions of the separatory funnel used in the test for air embolism

COMMENT

To illustrate the value of an adequate method I cite here 5 cases of air embolism which came to my attention recently

CASE 1—A 39 year old Negro man was stabbed in the left side of the neck, with laceration of the left jugular vein. He returned to his home and collapsed suddenly. At autopsy, thirteen hours after death, air bubbles were demonstrated in the bloody fluid of the right ventricle and the pulmonary arteries.

CASE 2—Sudden death occurred in a 26 year old white woman after fenestration of the right ear with skin grafting. The new procedure of temporary withdrawal of blood to lower the blood pressure and subsequent retransfusion was

used.² A sudden complete respiratory and cardiac arrest developed. An autopsy was made two hours after death. The carotid arteries were ligated. Air bubbles were seen in the cerebral arteries and in the arteries of the retina when an ophthalmoscope was used. Air bubbles were demonstrated in the left ventricle, in the aortic arch and in the carotid arteries. There was almost no air in the right ventricle.

CASE 3—In the course of a surgical operation for torticollis of the right side of the neck of a 2 year old girl, the dome of the right pleura was slightly lacerated in a slitlike manner. The slit was closed by surgical procedure. Sudden collapse and death occurred. At autopsy, three hours after death, a diagnosis of acute emphysema of the anterior mediastinum and bilateral pneumothorax (more marked on the right side) was made. There was acute dilatation of the right cardiac ventricle. Air was demonstrated in both pleural sacs and in the mediastinum.

CASE 4—A 27 year old white woman was found dead on the floor of the rest room of a hotel. There were no visible signs of violence. At autopsy, about eleven hours after death, the diagnosis was air embolism following attempted abortion in a case of pregnancy of two and one-half months' gestation. A lesion of the mucosa in the left lateral corner of the uterus was discovered. The embryonic sac was intact. Air was demonstrated in the right ventricle and in the pulmonary arteries. Air bubbles were also demonstrated in the ovarian veins and in the venous spaces of the uterine wall.

CASE 5—A 47 year old white man had an infected right eyeball, which was severely damaged further during a fight. He was taken to a hospital, and there the eye was removed after anesthesia had been induced with thiopental sodium (pentothal sodium®) injected intravenously into the left arm. The widened ophthalmic veins were not ligated. The patient died suddenly after the completion of the operation. At the autopsy, nineteen hours later, air was demonstrated in the right jugular vein, the descending vena cava and the right cardiac ventricle. The resulting diagnosis of the cause of death was air embolism following enucleation of the right eye.

SUMMARY

A new device is presented to demonstrate the presence of air embolism, pneumothorax and like conditions. This device may also be used to trap gases for identification and analysis in cases of poisoning by volatile solvents and gases and might materially aid in the diagnosis of caisson disease. Five cases investigated recently are cited to illustrate the value of the device.

2 Harris, H. E., and Hale, D. *Tr Am Acad Ophth* 52 90, 1947

Books Received

DIAGNOSTIC PROCEDURES FOR VIRUS AND RICKETTSIAL DISEASES First edition
Price, \$4 Pp 347, with illustrations New York American Public Health
Association, 1948

This volume is a response to the increasing demand for a collection of laboratory methods of diagnosis of virus and rickettsial diseases in man. The authors are a committee, with Francis Thomas Jr, as chairman, of the American Public Health Association. Diagnostic procedures for the following diseases are described by investigators in virus and rickettsial infections: psittacosis, K F Meyer and B Eddie, lymphogranuloma venereum, G Rake, trachoma and inclusion blenorrhea, P Thygesen, variola and vaccinia, R F Parker, influenza, G K Hirst, primary atypical pneumonia, A E Feller, mumps, J F Enders and J H Levens, poliomyelitis, J R Paul, encephalitis, W M Hammon, rabies, H N Johnson and T F Sellers, herpes simplex, T F McNair Scott, yellow fever, J C Bugher, dengue, A B Sabin, rickettsial diseases, N H Topping and J E Smadel. "Conceived as a manual for the laboratory worker and student the book is not intended to be a handbook of clinical diagnosis or of theoretic virology. It is a trial flight of a limited nature which the committee in future revisions will undoubtedly expand and improve as experience indicates."

BONE MARROW BIOPSY Haematology in the Light of Sternal Puncture By S J Leitner, M D, reader in internal medicine, University of Berne (Switzerland), and deputy medical superintendent, Sanatorium for Tuberculosis, Heilingschweni, Berne. English translation, revised and edited by C J C Britton, M D, Ch B, D P H, consulting haematologist to the Prince of Wales's General Hospital, Tottenham, London, and Queen Mary's Hospital, Roehampton, and E Neumark, M B, B S (London), M R C S, L R C P, lecturer in pathology, St Mary's Hospital Medical School, London. Price, \$8.50 Pp 433, with 7 plates (6 in color) and 194 text figures. New York Grune & Stratton, 1949.

Since biopsy of marrow has become a routine procedure in many hospitals an authoritative book on this subject should be welcome. Such a text should help the novice to gain the necessary experience and at the same time provide the expert with a reliable and comprehensive review of the many problems related to the interpretation of the findings in the disorders affecting the hemopoietic system. In general, Leitner's book covers the ground well. The technic of sternal puncture is adequately discussed, but the modern methods of hip and spinous process punctures are not mentioned. The characteristic marrow patterns are well described, and numerous case histories illustrate the diagnostic significance of biopsy of the marrow. However, the novice will regret the scarcity of colored pictures and will find it difficult to visualize important details from the black and white photomicrographs. The expert in this country* will be delighted with the many aspects and references pertaining to the continental literature. He will regret that the covering of the American contributions is incomplete and thus renders this work less valuable to him than it could have been. Although this book can be recommended for the hematologic library, there is still a need for a better international text on this subject.

UNUSUAL MALFORMATION OF THE LEFT ATRIUM PULMONARY SINUS

ERNST LOEFFLER, M D †
CHICAGO

A RARE anomaly of the left atrium of the heart is presented. This anomaly—in the literature referred to as triatrial heart—was found incidentally at the necropsy of a 70 year old Negro woman, who died four days after being admitted to Cook County Hospital with the clinical diagnosis of hypertensive heart disease and grade 4 decompensation. A detailed report of the necropsy is omitted, since it merely confirmed the clinical diagnosis. The cardiac malformation had no bearing on the clinical picture or the cause of death.

The heart was enlarged, weighing 600 Gm (body weight, 94 Kg). The apex was formed by both ventricles. The left ventricular wall was 19 mm, the right 7 mm, thick. The mitral ostium admitted two fingers freely, measuring 10.5 cm in circumference. The line of closure was slightly thickened, and the mitral ring was partly calcified. The chordae tendineae were not shortened. The aortic ostium measured 7.5 cm, the pulmonary ostium 8.5 cm, in circumference.

The left atrium was almost completely subdivided into an anterior and a posterior part. These two compartments communicated by an almost circular opening close to the left margin of the heart above the attachment of the posterior cusp of the mitral valve. The partition was achieved by a fold originating just to the left of the atrial septum on the posterior wall of the atrium and from its roof. The fold protruded convexly into the anterior part of the atrium, it consisted of a firm plate of connective tissue covered by thin endocardium. The fold ended with a sharp sickle-shaped border facing downward and to the left, bounding the communication between the posterior and the anterior half of the left atrium. The anterior part consisted of a wide central space, into which the posterior part opened. Posteriorly, the anterior chamber extended as a narrow slit between the described partition and the septum atriorum to the posterior wall of the heart. The atrial septum was normal, with a valve of the oval foramen visible on its left surface. The anterior compartment continued into a normally

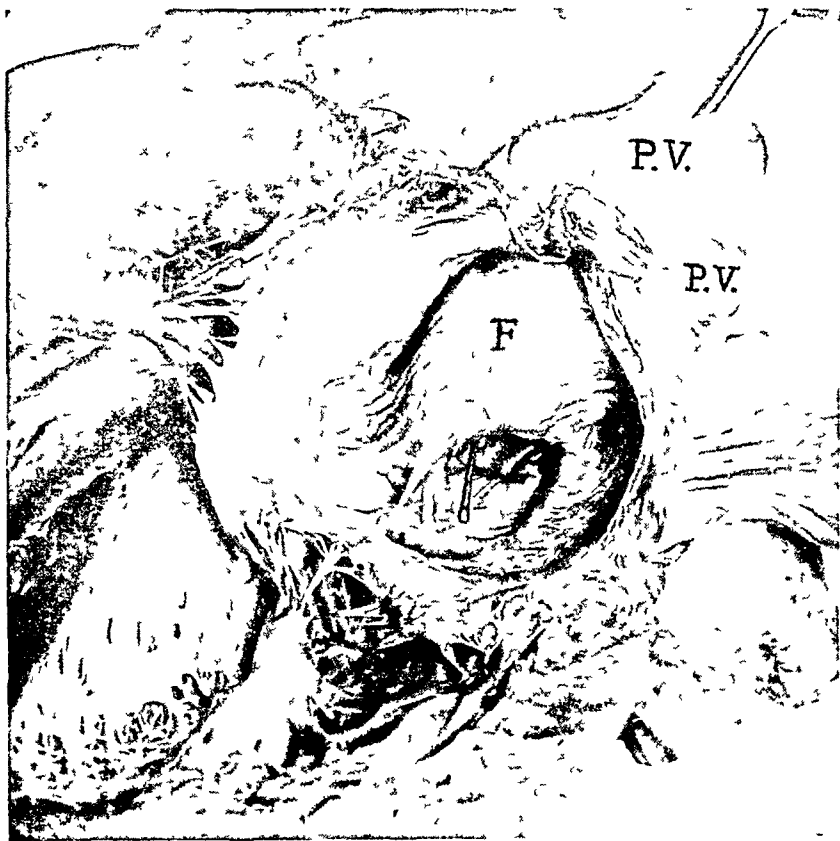
From the Department of Pathology and Hektoen Institute for Medical Research of the Cook County Hospital

† Dr. Loeffler died Sept. 24, 1949.

shaped auricular appendage and communicated with the left ventricle through a normal atrioventricular opening

The posterior compartment of the left atrium received the four pulmonary veins, which were in normal position. The posterior wall of the posterior compartment consisted of cardiac muscle.

Histologically, the fold consisted of dense connective tissue containing a few cardiac muscle fibers, between the collagenous bundles there were elastic fibers which formed a somewhat denser layer in the subendocardial layers.



Left atrium with fold. A probe has been introduced into the upper left pulmonary vein and through the posterior compartment of the left atrium. *P V* indicates left pulmonary veins, *F*, the dividing fold.

COMMENT

Previously reported similar cases, listed and reviewed in the papers of Patten and Taggart,¹ Palmer² and Pfennig,³ can be classified into three main groups.

1. In this group the left atrium is subdivided into two compartments by a diaphragm without any communication between the upper space

1 Patten, B. M., and Taggart, W. B. *Arch Path* 8: 894, 1929.

2 Palmer, G. A. *Am Heart J* 6: 230, 1930.

3 Pfennig, E. *Virchows Arch f path Anat* 307: 579, 1941.

receiving the pulmonary veins, and the lower, which carries the auricular appendage and opens into the left ventricle. In Hagenauer's^{3a} patient, a 4 month old baby, the posteriosuperior compartment communicated with the right atrium through a patent oval foramen. In Stoeber's⁴ patient, dying shortly after birth, part of the pulmonary veins entered the right atrium.

2 The second group comprises cases in which one or several small openings were found in the diaphragm (Potter and Ranson,⁵ Palmer,² Hosch,⁶ William and Abrikosoff,⁷ Faber,⁸ Borst^{8a} and Pfennig³). All those who had this type were infants or children, with the exception of Borst's^{8a} patient, a 38 year old kyphoscoliotic woman, who died with the symptoms of mitral stenosis, the opening in the dividing septum measured 1 cm.

3 The third group are cases presenting only partial subdivision of the left atrium, there were no clinical symptoms and no other cardiac changes referable to the malformation, they were discovered incidentally at necropsies (Church,⁹ Griffith,¹⁰ Fowler¹¹). The present case is of this type. In these hearts a fibromuscular band stretches through the left atrium. Behind and above this band the pulmonary veins open, and in front and below, the atrioventricular ostium is found. The bands are of variable width and may even be reduced to cords, described as false tendinous cords, as mentioned by Siegmund in a note to Pfennig's³ paper.

The different explanations offered by the authors may be grouped as follows:

1 The septum is said to be an overgrowth of the valve of the oval foramen (Fowler,¹¹ Potter and Ranson,⁵ Hosch,⁶ Preisz¹² and Griffith¹⁰). This theory can be easily rejected, since the unaltered foramen ovale can be seen in its proper place.

2 Borst's^{8a} theory is that of a primary displacement of the main pulmonary vein, the diaphragm is interpreted as the septum primum.

3a Hagenauer. *Frankfurt Ztschr f Path* **41** 332, 1931

4 Stoeber. *Virchows Arch f path Anat* **193** 252, 1908

5 Potter and Ranson. *J Anat & Physiol* **39** 69, 1904

6 Hosch, P. H. *Frankfurt Ztschr f Path* **1** 565, 1907

7 William and Abrikosoff. *Virchows Arch f path Anat* **203** 404, 1911

8 Faber. *Zentralbl f Path* **61** 224, 1934

8a Borst. *Zentralbl f Path* **16** 812, 1905

9 Church, W. S. *Tr Path Soc, London* **19** 188, 1867

10 Griffith, T. W. *J Anat & Physiol* **37** 255, 1902

11 Fowler, J. K. *Tr Path Soc, London* **33** 77, 1881

12 Preisz. *Beitr z path Anat u z allg Path* **7** 272, 1889

of Boïn, which, owing to an increased intensity of growth, formed the whole final atrial septum. The opening in the diaphragm is the ostium primum (Borst,^{8a} Stoeber,⁴ Patten and Taggart¹ and Faber⁸). All these assumptions are mere speculation and are contradicted by the presence of a regularly formed, though perforated, atrial septum with the limbus and the valve of the oval foramen in proper position. William and Abrikosoff⁷ accepted this theory of an anomalous primordium of the primary pulmonary vein but rejected that of the diaphragm as septum primum and the opening as ostium primum on the basis of their microscopic examination. The diaphragm is explained as the result of a splitting of the atrial septums due to the impact of the blood from the misplaced pulmonary vein.

3 The third theory, the principle of which I accept, is the following. The common pulmonary vein has not been incorporated in the left atrium as normally it would have been. The posterosuperior cavity, into which the pulmonary veins open, is the abnormally widened common pulmonary vein, best termed a pulmonary sinus. The septum or diaphragm, complete or incomplete, represents the posterior wall of the primitive left atrium. The first author to assume a failure of fusion of the primitive pulmonary vein and the left atrium was Griffith,¹⁰ one of the earliest describers of this malformation. However, he abandoned this theory for no other reason than that it did not meet with the approval of the members of the society in which he presented his case. Other followers are Palmer,² Hagenauer^{3a} and Pfennig.³

The mechanism by which the anomaly develops has been discussed by Hagenauer^{3a} and by Pfennig.³ The former assumed that the first manifestation of the anomaly went back to that stage of development of the heart in which, according to Spitzer, the two atriums are arranged in a distoproximal sequence. If the primitive pulmonary vein opens at an oblique angle into the distal atrium, an early closure of the opening may occur during the widening of the prospective left atrium. The overexpanded pulmonary vein would later gain a new opening into the right atrium. This explanation is obviously wrong, because the arrangement of the different segments of the heart, according to Spitzer's concept, represents a phylogenetic and not an ontogenetic stage. During the development of a mammalian and a human heart the two atriums are from the beginning arranged side by side. Pfennig,³ in his explanation of the mechanism of the development of the malformation, assumed that there was a primary occlusion of the primitive pulmonary vein and that this prevented its being incorporated into the left atrium. However, he stated that he did not know why this primary occlusion occurred.

From present knowledge of the development of the heart and the pulmonary vein and from a careful analysis of the cases reported in the literature, the following developmental mechanism is suggested

The pulmonary vein is at first a single blood vessel, opening into the left atrium close to the line at which the atrial septum is attached to the posterior wall. The single pulmonary vein originates from the confluence of a right and a left pulmonary vein, each composed of two main branches. Very soon the short common terminal part of the pulmonary vein is drawn out and incorporated into the posterior wall of the left atrium, so that in this stage right and left pulmonary veins have gained independent openings into the heart. While the part of the wall of the left atrium between the right and left pulmonary veins grows at considerable speed, the process by which the veins are incorporated into the atrium continues until the common terminal parts of the two left and the two right pulmonary veins also have become part of the left atrium. In this stage the final relations of the left atrium have been established. Its posterior wall is almost in its entirety derived from the wall of the pulmonary vein.

The primary malformation seems to be a failure of the single pulmonary vein to be incorporated into the left atrium, caused in all probability by a disturbance of the normal growth of the posterior wall of the left atrium. The next consequence is a growing disparity between the widening primitive pulmonary vein and its narrow opening into the left atrium. From now on several possibilities of compensatory changes must be considered. The pulmonary vein, bulging against the right atrium, may gain an opening into the right atrium and either the normal left atrial communication may remain as a small defect or the "septum" may close entirely. In the latter case a complete septum will be found between the pulmonary sinus and the left atrium. The sinus opens into the right atrium, and as a further consequence the oval foramen remains patent. In other patients the original opening of the single pulmonary vein experiences early enough a secondary widening, through which later the pulmonary sinus empties its blood into the left atrium. The septum atriorum develops normally, with the limbus and the valve of the oval foramen in normal shape and relation. Finally the widening of the opening of the pulmonary vein into the left atrium may be followed by a dehiscence of the septum between the pulmonary sinus and the left atrium, so that the pulmonary sinus is incompletely separated from the left atrium by a fibromuscular band.

In none of the reported cases had the condition been suspected during life. If the anomaly is not combined with other malformations, the clinical signs should be those of mitral stenosis. Summaries are

found in the papers of Abbot,¹³ Poynter,¹⁴ and Bredt,¹⁵ who reviewed congenital malformations of the heart more from the anatomic point of view. It is not mentioned in Taussig's¹⁶ book, which serves primarily clinical interests.

The first author to describe a malformation such as the one reported here was Church⁹, the first to mention it was Andral,¹⁷ who listed it as supernumerary atrium under the heading of "exces de développement du cœur."

SUMMARY

A pulmonary sinus within the left atrium is described, and the malformation is explained by failure of the primary pulmonary vein to be incorporated into the left atrium, owing to a disturbance of the normal growth of the posterior atrial wall. The designation "heart with pulmonary sinus" is suggested instead of "triatrial heart" for malformations of this type.

13 Abbot, M. E., in *Nelson's Loose Leaf Medicine*, New York, Thos. Nelson & Sons, 1946, vol. 4, p. 207.

14 Poynter, C. W. *Congenital Anomalies of the Heart*, Lincoln, Nebraska, University Studies, 1919.

15 Bredt. *Ergebn. d. Path.* **30** 106, 1936.

16 Taussig, H. B. *Congenital Malformations of the Heart*, New York, The Commonwealth Fund, 1947.

17 Andral, G. *Precis d'anatomie pathologique*, Paris, Gabon, 1929, vol. 2, p. 313.

APICAL PNEUMONIC SCARS

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IN THE past all apical scars have been considered the result of the action of either the tubercle bacillus or silica. As to the proper division of the scars between these two agents opinions have differed. Some scars are undoubtedly due to silica and others to the tubercle bacillus, but the great majority of apical scars have a characteristic and constant structure by which they may be distinguished from either of the other types. The purpose of this study is to stress the differences between the various types of apical scars and to discuss the cause of the commonest one.

Davson and Susman¹ divided all apical scars into two types, A and B. Type B scars are those which are clearly tuberculous because of the presence of scarred or caseous tubercles. Equal proof is not available for the authors' contention that the commoner type A scars are due to the accumulation of silicious dust. Most of them do contain considerable amounts of silica but, as Belt, Irwin and King² have pointed out, the lymphatic channels are obstructed in fibrous scars, and inhaled dust will tend to accumulate in these areas. Some otherwise representative type A scars have little silica in them, and Davson and Susman¹ circumvented this difficulty by suggesting that an inhaled irritant other than silica may be responsible.

More recently, Medlar³ has reported the results of a macroscopic study of apical scars encountered in a series of 1,259 autopsies. He found that in 106 persons under 20 years of age no such scars were present. The incidence of tuberculosis in this group was 21.7 per cent. In 403 white men over 50 years of age, scars were present in 68.2 per cent, and the lesions were bilateral in 98 per cent. Medlar concluded from this and other observations that apical scars are not etiologically related to tuberculous infection.

To obtain material for the study of this question pulmonary apices were examined microscopically in a series of 40 autopsies.

From the Department of Pathology, Toronto University.

1 Davson, J, and Susman, W. *J Path & Bact* **45** 597, 1937.

2 Belt, T H, Irwin, W A, and King, E S. *Canad M A J* **34** 125, 1936.

3 Medlar, E M. *Am Rev Tuberc* **55** 511, 1947.

METHOD

If a scar was visible, it was excised and sectioned. Otherwise, a block was taken from the apex in the hope of finding microscopic scars. Three consecutive sections were then cut. One was stained with hematoxylin and eosin, one with Weigert's elastic tissue stain and Van Geison's stain and the third was incinerated and treated with hydrochloric acid by the method described by Irwin⁴. It is obviously easy to miss small tubercles by this method, and therefore, in 4 cases in which on examination of the three sections the scar was classified as nontuberculous, the whole lesion was reexamined by serial section. Every twentieth section was stained and examined.

RESULTS

In the 40 autopsies 17 scars were discovered. One scar was definitely tuberculous. One scar showed nodular silicosis. The remaining 15 were classified in a group by themselves, but 1 of them was transferred into the tuberculous category after serial sections had been made because of the finding of a small healed tubercle. This left 14 scars so similar that one description will serve for all. The pleura was usually not thickened over the scar. The structure was surprisingly uniform, regardless of size in all instances. The alveolar walls stood out in sections stained with hematoxylin and eosin as wavy, brightly stained, eosinophilic, slightly refractile bands of elastic tissue. The alveolar spaces were moderately collapsed and contained pale-staining fibrous tissue composed of thin, parallel strands (fig 1). Large, dilated bronchi filled with exudate were often present. Branches of the pulmonary artery were narrowed by endarteritis obliterans. Anthracotic pigment was scattered throughout in varying quantities but never in nodular clumps. The distinguishing characteristics were the uniformity and the clearly outlined alveoli filled with fine strands of fibrous tissue. In the sections stained for elastic tissue (fig 2), the alveolar walls were clearly outlined. The elastic fibers had contracted and stained deeply.

There was a marked variation in the amount of deposit left after the incineration and hydrochloric acid treatment. The amount of silica paralleled the amount of anthracotic pigment throughout the remaining lung and the pigment in the scar itself. There was no relation between the concentration of the silicotic residue and the amount of fibrous tissue in the scar. The deposit was scattered at random and not in nodules. Sometimes the amount of silica was very small, but in no scar was it lacking.

COMMENT

The possible etiologic factors responsible for these fourteen scars can conveniently be discussed under several headings, and many possible causes of apical scarring can be ruled out.

Classic Tuberculosis—The absence of follicular lesions, nodular fibrosis or caseation rules out the possibility of fibrocaseous tuberculosis.

Exudative Tuberculosis—According to Jaffé,⁵ pulmonary scars may be produced by nonresolution of an exudative type of tuberculosis in the absence of true tubercle formation or caseation. The fact that in 1 of the 4 cases in which a scar was sectioned serially the lesion

4 Irwin, W. A. *Canad. M. A. J.* **31**: 135, 1934.

5 Jaffé, R. H. *Arch. Path.* **18**: 712, 1934.

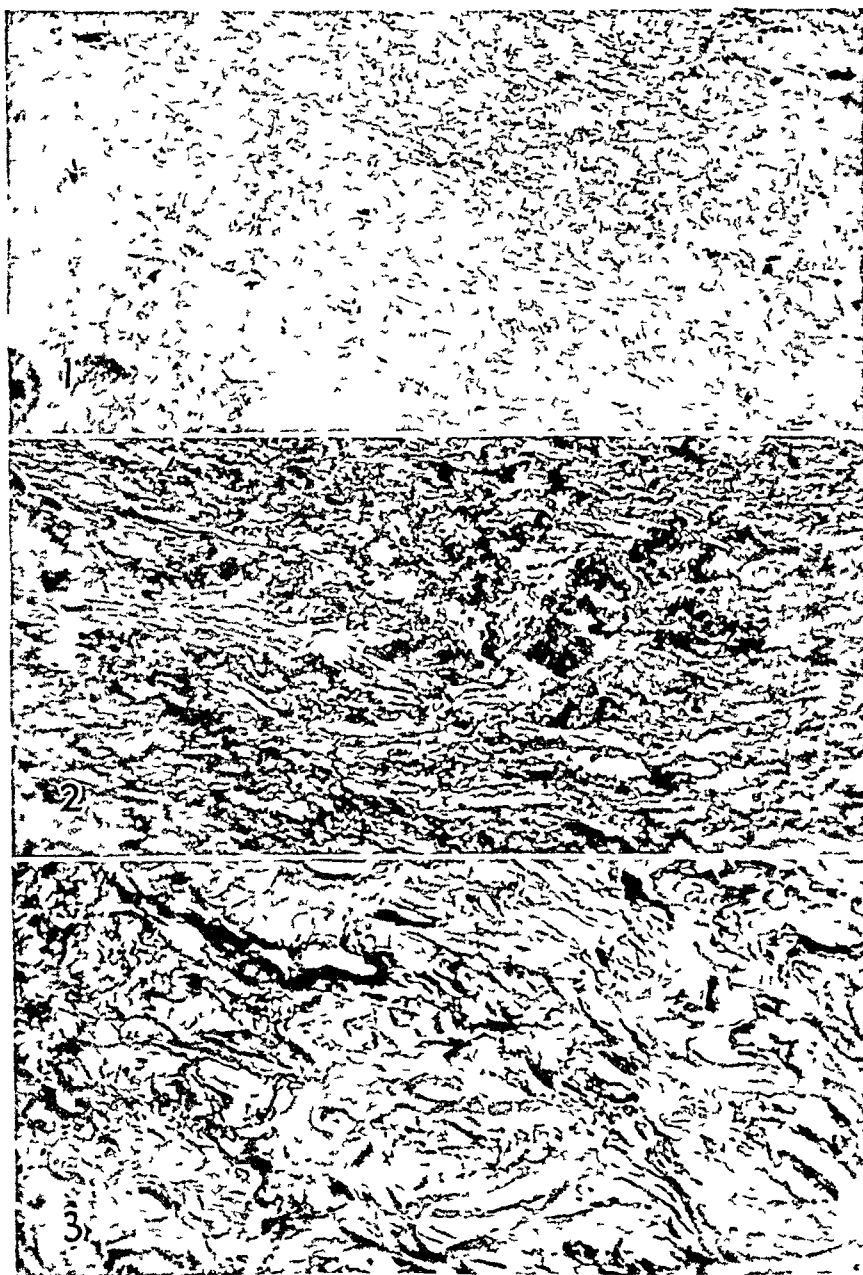


Fig 1—Photomicrograph of an apical scar. The alveolar walls are intact, and fine fibrous tissue fills the alveoli. Hematoxylin and eosin, $\times 67$.

Fig 2—Photomicrograph of the same scar. The alveolar walls are clearly visible. Weigert's elastic stain, $\times 67$.

Fig 3—Photomicrograph of an area of fibrosis outside a silicotic nodule in the apex. Broad bands of collagen cut the elastic tissue into fragments. Weigert's elastic stain, $\times 180$.

had to be transferred to the tuberculous group raised the question whether all these scars had a tuberculous origin. The statistics produced by Medlar³ on age incidence of apical scarring and tuberculosis make this unlikely. There is a possibility that in the case in question tuberculosis was a complicating factor in the more usual type of scar. Undoubtedly, if all the scars had been sectioned serially, more tubercles would have been found.

Nodular Silicosis—To quote from a recent paper of Costero's⁶

The histological basis of all lesions induced in the human lung by silicious dust is the proliferation of reticular fibers. After proliferation these fibers are partially or wholly transformed into collagen and undergo hyalinization and retraction.

The elastic fibers disappear in the completely constituted lesions. Their disposition outside the latter is indicative of traction initiated by shrinkage.

Figure 3 is a photomicrograph of a field outside a silicotic nodule. Broad bands of collagen cut the elastic tissue into fragments. The contrast between it and the common type of apical scar (figs 2 and 3) is striking.

Silicotic Reticulosis—In the early reticular lesions of silicosis there is thickening of the periarterial connective tissue by dust and reticulin fibers. Surrounding alveoli are compressed, and there is no development of fibrous tissue in the alveolar spaces.

Acute Infarction—Recent infarcts are not commonly observed in the apex, and acute infarction does not occur in otherwise normal lungs. The high incidence of apical scars would therefore be difficult to explain on the basis of infarction. Furthermore, the elastic pattern is not preserved in a healed infarct.⁷

Chronic Infarction—It has been mentioned that branches of the pulmonary artery are narrowed in these scars. Dock⁸ pointed out that the effective pulmonary artery pressure at the apex in the erect posture is almost nil. It must be considered whether this in itself could produce an obliterative endarteritis of the pulmonary artery and consequent slow ischemic scarring of the apex. The histologic picture would still not correspond.

Collapse—It is an established fact that respiratory movement is minimal at the apex. In older people who, because of sedentary habits of occupation and relaxation, never inspire deeply, the collapse of a few apical alveoli is easy to visualize. If then fibrous strands were to develop in the alveoli, this collapse would be made permanent and a typical scar would be produced. However, respiratory movement is equally poor along the posterior lung border close to the hilus,

6 Costero, I. *Am J Path* **24** 49, 1948.

7 Castleman, B. *Arch Path* **30** 132, 1940.

8 Dock, W. *Am Rev Tuberc* **53** 297, 1946.

and similar scars are not found in this location. Parenchymal scarring is also not a complication of therapeutic collapse.

Pneumonia—Castleman⁷ described these scars as the result of organized tuberculous pneumonia. For the reasons already given, I do not agree that the pneumonia is invariably tuberculous. From consideration of the histologic characteristics, I think that the scars are the result of a low grade inflammation. The incidence of lobar and bronchopneumonia is not high enough to make them likely causes. Primary atypical pneumonia, however, is very common and may involve the apex, it is possibly the prime factor in many of these scars. The peculiar localization of the scarring is probably dependent on two factors: poor apical movement and a very low effective arterial pressure. The important fact is that these scars are the result of an old pneumonia and in most cases not a tuberculous pneumonia.

SUMMARY

Apical scars were collected for microscopic study from a series of autopsies. Three types of scars were found, tuberculous, silicotic and a third type, which was the commonest. The common type of apical scar, in which the alveolar pattern remains intact, is the result of an organized pneumonia which is in most cases not tuberculous.

COARCTATION OF THE AORTA WITH DEATH FROM RUPTURE OF A CEREBRAL ANEURYSM

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THE ASSOCIATION of coarctation of the aorta and cerebral aneurysm is an interesting one. The following case, published with the permission of Prof S J Hartfall, is the sixteenth recorded and the eleventh in which death occurred from rupture of the aneurysm, both coarctation and cerebral aneurysm being demonstrated at autopsy in each case.

REPORT OF A CASE

P M, a single woman aged 19, while at work in a clothing factory was seized with severe headache and vomiting, she then collapsed, and died about nine hours later. The previous day she had complained of slight headache but had otherwise been perfectly well. Her mother described her as a bright girl who was always on the move, the most active of her seven children. This girl had led an energetic life, had played games while at school, was later in the Women's Land Army and went dancing nearly every evening. When excited, it was particularly noticed, she became red in the face. At the age of 2 she had whooping cough, at which time a doctor had remarked that she had a "strained heart".

On being admitted to the Leeds General Infirmary she was found to be drowsy with a pale ashen face and cyanosed lips, but could be roused by persistent questioning. There was urinary incontinence.

Examination revealed rigidity of the neck and Kernig's sign. The pupils reacted to light, and the fundi showed multiple hemorrhages. Muscular tone was diminished in the left leg, the left knee jerk was absent, and there was left ankle clonus. Lumbar puncture showed a pressure of spinal fluid of 100 mm of water, rising freely on compression of the jugular vein, the fluid was blood stained. The pulse was completely irregular, with a rate of 98. The apex beat of the heart was situated $4\frac{1}{2}$ inches (11.5 cm) from the midline and was irregular, with a low-pitched diastolic murmur, the first and second aortic and pulmonary sounds were split.

Subarachnoid hemorrhage, probably due to ruptured aneurysm, was diagnosed.

The coarctation was overlooked, not surprisingly, since the serious cerebral complication made careful examination difficult.

Autopsy—The body was that of a spare young woman, weighing 91 pounds (41 Kg). The heart weighed $10\frac{1}{2}$ ounces (297.5 Gm), and there was some hypertrophy of the left ventricle. The myocardium was firm and healthy looking. There was a congenital bicuspid aortic valve, one cusp taking the place of the anterior and left posterior cusps. A slight valvular patency of the foramen ovale was present. The coronary arteries showed patches of atheroma without any narrowing. The aorta was normal in size up to a point just beyond the origin of

the left subclavian artery, where it rapidly narrowed to a diaphragm-like stenosis with a small central opening 2.5 mm in diameter. Proximal to this coarctation, atheromatous streaking was evident in the aorta and was particularly well marked in the common carotid arteries. Immediately below the constriction the aorta widened to a caliber only slightly less than that of the proximal part. The abdominal aorta was hypoplastic, especially below the origin of the renal arteries. There was no atheroma distal to the stenosis.



Fig 1—Coarctation of the aorta and proximal atheroma, the latter especially evident in the common carotid arteries, $\times 0.80$

The brain showed some flattening of the cerebral convolutions. There was extensive subdural hemorrhage over both hemispheres, with subarachnoid hemorrhage at the base, particularly around the pons and between the frontal lobes. Situated on the left anterior cerebral artery at its junction with the anterior communicating artery there was a small aneurysmal sac embedded in recent blood clot. The aneurysm in its collapsed state measured about 0.7 cm in diameter and had ruptured on its deep aspect. The hemorrhage had caused much destruction

of the adjacent frontal lobes and on the left side had entered the lateral ventricle. The basal ganglions on both sides showed petechial hemorrhages. The vessels at the base of the brain showed no sign of atheroma, it is most probable that the aneurysm was of a developmental type.

The other organs showed no notable changes and appeared healthy.

COMMENT

Coarctation of the aorta is not a common lesion. From the time of the earliest reported case in 1791 up to 1928 Abbott¹ collected 200 cases of the "adult type" in which autopsies had been made, and since the latter date Reifenstein, Levine and Gross² have reviewed a further 104 cases up to 1947, making in all 304 recorded cases.

In 10 cases of coarctation confirmed at autopsy death was found to be due to rupture of a clearly defined cerebral aneurysm. Five of these cases occurred before 1928 and were briefly reviewed by Abbott. They were recorded by Eppinger (1871, case 2), Kolisko (1913, case 2), Strassman (1922, case 7), Woltman and Shelden (1927, case 1) and Parkes Weber (1927). Since this review 5 further cases have been reported by the following authors: Green³ (1928, case 3), Bode and Knop⁴ (1929, case 2), Forster⁵ (1940), Bramwell and Jones⁶ (1941) and O'Reilly and Chapman⁷ (1943).

Analysis of these cases, including the present one, reveals that in 4 of the 11 there was more than one aneurysm present. As noted by Woltman and Shelden,⁸ these patients tended to be young and vigorous, and rupture of the aneurysm was evidently the immediate result of physical strain, as in the present case. Abnormally high blood pressure must have been present in every case, as hypertrophy of the left ventricle was found in each. The average age was 23 years, the youngest patient was 13 and the oldest 40, and 9 of the patients were males. The ruptured aneurysms ranged in size from a hempseed to a walnut, and it is interesting to note that the largest aneurysms were found in the oldest patients, both patients aged 40 had large aneurysms. The other patients were all under 26 and had small aneurysms with

1 Abbott, M. E. *Am Heart J* **3** 574, 1928.

2 Reifenstein, G. H., Levine, S. A., and Gross, R. E. *Am Heart J* **33** 146, 1947.

3 Green, F. H. K. *Quart J Med* **21** 419, 1928.

4 Bode, O. B., and Knop, F. *Deutsches Arch f klin Med* **163** 298, 1929.

5 Forster, A. *Deutsche Ztschr f d ges gerichtl Med* **33** 115, 1940.

6 Bramwell, C., and Jones, A. M. *Brit Heart J* **3** 205, 1941.

7 O'Reilly, J. N., and Chapman, O. W. *Arch Dis Childhood* **18** 109, 1943.

8 Woltman, H. W., and Shelden, W. D. *Arch Neurol & Psychiat* **17** 303, 1927.

the exception of Green's patient whose aneurysm was 3 cm in diameter. All the ruptured aneurysms arose from intracranial branches of the internal carotid arteries.

A further case of coarctation and cerebral aneurysm confirmed at autopsy was recorded by Davies and Fisher⁹ (1943). Their patient, a youth aged 17, died of a ruptured aorta, and a small aneurysm with evidence of previous leakage was found on the left middle cerebral artery.



Fig 2—Ruptured cerebral aneurysm at the junction of the left anterior cerebral and anterior communicating arteries, $\times 35$

Four cases are recorded in which coarctation and an unruptured cerebral aneurysm were found at autopsy, the patient dying from some other condition. In all 4 the aneurysm involved either the basilar or one

⁹ Davies, J. N. P., and Fisher, J. A. *Brit Heart J* 5 197, 1943

of the vertebral arteries Abbott, in her review, mentioned 2 of these cases, those of Knierim (1880, basilar) and Sommerbrodt (1883, vertebral) Boyd and Werblow¹⁰ recorded a further case (vertebral) in 1938, and Walker and Livingstone¹¹ (case 2) the fourth (vertebral) also in 1938 In the last instance subarachnoid hemorrhage had occurred, but although the left vertebral artery showed irregular saccular aneurysmal dilatations no definite rupture was found, the patient also had infective endocarditis

Walsh and King¹² (1942, case 4) recorded a case of rupture of an aneurysm of the anterior communicating artery confirmed by post-mortem examination Probable coarctation of the aorta was recorded on clinical examination, but there is no mention of confirmation of this diagnosis post mortem

Lichtenburg and Gallagher¹³ in 1933 reported a case of coarctation of the aorta associated with intermittent leakage of a cerebral aneurysm, diagnosed during life Their patient, a girl aged 12, was still living after eighteen months' observation These authors could find in the literature no record of a similar condition diagnosed in life, but a case was later recorded by Baker and Shelden¹⁴ (1936), that of a woman of 25, and a further case by Davies and Fisher (1943), the already mentioned case of a youth aged 17 In the last case the diagnosis of subarachnoid hemorrhage was confirmed by lumbar puncture and that of aneurysm by postmortem examination thirteen months later, after the patient had died of a ruptured aorta

SUMMARY

An active, healthy girl of 19 collapsed suddenly and died and was found at autopsy to have a ruptured cerebral aneurysm associated with coarctation of the aorta Ten cases with similar findings have been found recorded in the literature, together with 1 case of leaking aneurysm and 4 of unruptured aneurysm, making a total of 16 cases of coarctation associated with cerebral aneurysm in which the diagnosis was confirmed post mortem A further 3 cases are recorded but without post-mortem confirmation

10 Boyd, L J, and Werblow, S C *Ann Int Med* **11** 845, 1938

11 Walker, J B, and Livingstone, F D M *Lancet* **2** 660, 1938

12 Walsh, F B, and King, A B *Arch Ophth* **27** 1, 1942

13 Lichtenburg, H H, and Gallagher, H F *Am J Dis Child* **45** 1253, 1933

14 Baker, T W, and Shelden, W D *Am J M Sc* **191** 626, 1936

SEVERE ADRENAL CORTICAL ATROPHY (CYTOTOXIC)
AND HEPATIC DAMAGE PRODUCED IN DOGS
BY FEEDING 2,2-BIS(PARACHLOROPHENYL)-
1,1-DICHLOROETHANE (DDD OR TDE)

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AND

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FOLLOWING the feeding of the insecticide 2, 2-bis(parachlorophenyl)-1,1-dichloroethane, commonly called DDD or preferably TDE, to dogs for periods of one to thirty-three months there was observed on both gross and microscopic pathologic examination an unusually consistent and severe atrophy of the adrenal cortex of the type generally designated as cytotoxic. Such a lesion has not been seen in the microscopic examination of over 300 other dogs in this laboratory following the usually prolonged feeding of about fifty other chemical compounds. Some of the compounds had produced severe damage of the liver or other organs, but rarely had they affected the adrenal glands in any manner, and none had caused adrenal lesions similar to those reported here. In particular, the closely related compounds DDT (2,2-bis(parachlorophenyl)-1,1,1-trichloroethane), methoxychlor and DDT dehydrochloride when administered to dogs in the same manner as TDE did not affect the adrenal glands. As stated in a preliminary note,¹ this is a striking example of chemical specificity in the causation of damage of an organ.

MATERIALS AND METHODS

Eleven young adult dogs were fed TDE dissolved in corn oil in capsules at levels of 50 to 200 (usually 50 or 80) mg per kilogram per working day. The dosage levels were kept constant. Seven of the dogs were mongrels and 4 were Irish terriers, 6 were female and 5 were male. Gross and microscopic pathologic examination was done on each of the 7 dogs that died and the 3 that were killed. One dog is still living and in apparent good condition thirty-eight months after the beginning of the experiment.

From each of the 10 dogs studied, hematoxylin-eosin stained paraffin sections of formaldehyde-fixed tissue were made from heart, liver, gallbladder, spleen, lymph nodes, pancreas, kidney, adrenal gland, thyroid gland, parathyroid gland,

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency

1 Nelson, A. A., and Woodard, G. Federation Proc. 7: 277, 1948

hypophysis, ovary (or testis) and uterus (or prostate), also frozen sections of kidney and liver were stained for fat with oil red O, and a Wright-Giemsa-stained smear of the bone marrow was made. Paraffin sections of lung and stomach and a frozen section of adrenal gland stained for fat were made from 9 dogs, and paraffin sections of thigh muscle, small intestine, colon, urinary bladder, rib bone, bone marrow and four levels of brain were made from 8 dogs. In several instances each, adrenal gland, liver and kidney were also fixed in Zenker's and/or Helly's fluid, and a Mallory type of differential connective tissue stain was made.

EFFECTS OBSERVED

Dosage of TDE, duration of feeding, etc., are given for each dog in the table. "M" in the dog number indicates that the animal was a mongrel, the others were Irish terriers. Changes resulting from treatment observed in the dogs during life were relatively slight. There was generally slight or moderate loss of weight, up to as much as 25 per cent of the initial weight. Much of this loss occurred in the last week or so of life, coincident with weakness and anorexia, which came on

Experimental Conditions for Individual Dogs

Dog	Sex	Age at Death, Mo	Time on Experiment, Mo	Dosage of TDE, Mg per Kg per Day	Weight at Start, Kg	Weight at Finish, Kg	Manner of Death
M 200	M	17	2	200	11.5	8.6	Died
M 198	M	16	2½	100	11.5	9.8	Killed
90-219	F	16	2½	80	9.9	7.7	Killed
M 228	M	(?)	1	80	7.0	6.0	Died
M 225	F	12	4	80	9.5	7.6	Died
M-196	M	33	20	50	9.5	9.0	Died
82-199	F	51	17	80	8.2	8.0	Died
M 201	F	38	21	50	8.3	8.3	Died
82-198	M	56	21	50	10.4	10.4	Killed
M 202	F	51	33	80	7.5	7.3	Died
90-218	F	Alive	38	50	11.0		

relatively suddenly. There were no convulsions or other neurologic symptoms. No pigmentary changes were noted.

GROSS PATHOLOGIC CHANGES

Slight or moderate emaciation was present in some of the dogs, corresponding to the loss of weight noted in these animals during life.

The adrenal glands of all the dogs, including the dog killed while apparently in good condition, were distinctly reduced in size. On section, this was accounted for by marked thinning of the cortex, which was also a deeper yellow than usual. The medulla appeared unaffected. The adrenal glands of dogs 82-198, M-201 and M-202, after formaldehyde fixation, were carefully trimmed of surrounding connective tissue and weighed, the respective combined weights were 0.50, 0.47 and 0.48 Gm. The adrenal glands of 3 control dogs of similar weight gave values of 0.82 Gm (2 dogs) and 0.87 Gm (1 dog) after similar treatment. Baker's² figures for the mean adrenal weights of dogs weighing 8.6 Kg (average weight of the 3 TDE dogs at the time of death) are considerably higher, namely 1.14 Gm for females in diestrus and 1.12 Gm for mature males. The variation between different observers in the degree of careful trimming of extra-adrenal tissue, a rather time-consuming chore, is probably a large factor in such differences of weight.

² Baker, D. D. *Am J Anat* 60:231, 1937.

The liver in most instances had a moderate or marked nutmeg appearance, with a yellowish background contrasting with the dark red lobular centers. The two exceptions were dogs 82-198 and 82-199, affected respectively with slight and moderate hepatic cirrhosis, about which more will be said under "Comment."

The kidneys of several dogs showed on section prominent whitish yellow to light orange fine radial streaking of the cortices, an accentuation of the fainter streaking normally present. Apart from this, the kidneys were normal in appearance.

Organs other than the adrenal gland, liver and kidneys showed no consistent gross changes. A few incidental lesions, each occurring in only 1 dog and of uncertain relation to the treatment, will be considered together with their microscopic appearances under that heading.

MICROSCOPIC PATHOLOGIC CHANGES

Adrenal Gland—Like the macroscopic appearance, the microscopic appearance of the adrenal gland was uniform throughout this series of dogs. Such slight variations of the microscopic picture as were present appeared to be related to the differing ages of the lesions, there was little variation in intensity.

The adrenal cortex was strikingly reduced in width, being in all instances no greater than about half the usual thickness, while in the extreme examples the thickness was about one-fifth the normal (figs 1 *B*, *C* and *D*). The normal structure was highly disorganized (figs 2 *A*, *B* and *C*). The zona glomerulosa was the best retained of any of the cortical zones, but it showed at least slight (in the dog killed while in apparent good health) and often marked changes in the form of loss of outline of the zone and of enlargement, rounding and foaminess of the individual cells. The zona fasciculata was shortened, the individual cords were irregular, and the cells showed the same types of alteration as did those of the zona glomerulosa. The zona reticularis had essentially vanished. Because of the loss of glandular cells from the inner portion of the cortex, there was usually an appearance of fibrosis in the juxtamedullary region, looser in the earlier examples and denser in the later ones. However, collagen stains of the Mallory type showed that most of this apparent fibrosis could be accounted for by condensation of the preexisting stroma.

Cortical inflammatory or necrotizing phenomena were never massive, but enough were present to suggest a slow, continuing process of damage with at least some coincidental attempt at repair. More of these changes were seen with the shorter than with the longer periods of survival. In the outer part of the cortex particularly, individual cells or small groups of cells showed fragmentation or pyknosis of the nuclei and/or oxyphilia or partial loss of the cytoplasm. At the same time small, undifferentiated cells appeared to be enlarging and differentiating into cortical cells. Other histologic features indicating a process other than simple atrophy were the irregularities of size and shape already noted in the individual cells and cell cords, and a mild degree of mobilization of small mononuclear and rare polymorphonuclear cells. No unquestionable mitotic figures were seen, and nothing even resembling adenomatoid hyperplasia was present. In the dogs surviving for the longer periods, the innermost cortical cells became even foamier and more like macrophages in appearance than previously and contained in addition to much lipid material a small amount of finely divided, light brown pigment.

Vascular abnormalities in and around the adrenal glands were looked for and were absent. In frozen sections the content of sudanophilic material and of doubly refractile material in such cortical cells as remained was not diminished. The adrenal medulla was uniformly uninvolved and gave its usual chromaffin reaction.

with fixatives containing potassium bichromate. Accessory cortical tissue is rare in dogs and was not noted in any location in this study.

Liver—Moderate or severe fatty degeneration was present in the liver of every one of the 10 dogs except the one killed while in apparently good condition. Moderate centrilobular atrophy and more or less centrilobular congestion were generally present, the congestion was apparently secondary to the atrophy of hepatic cells, since elsewhere in the body evidences of chronic congestion were absent. Present in from one third to two thirds of the dogs were slight

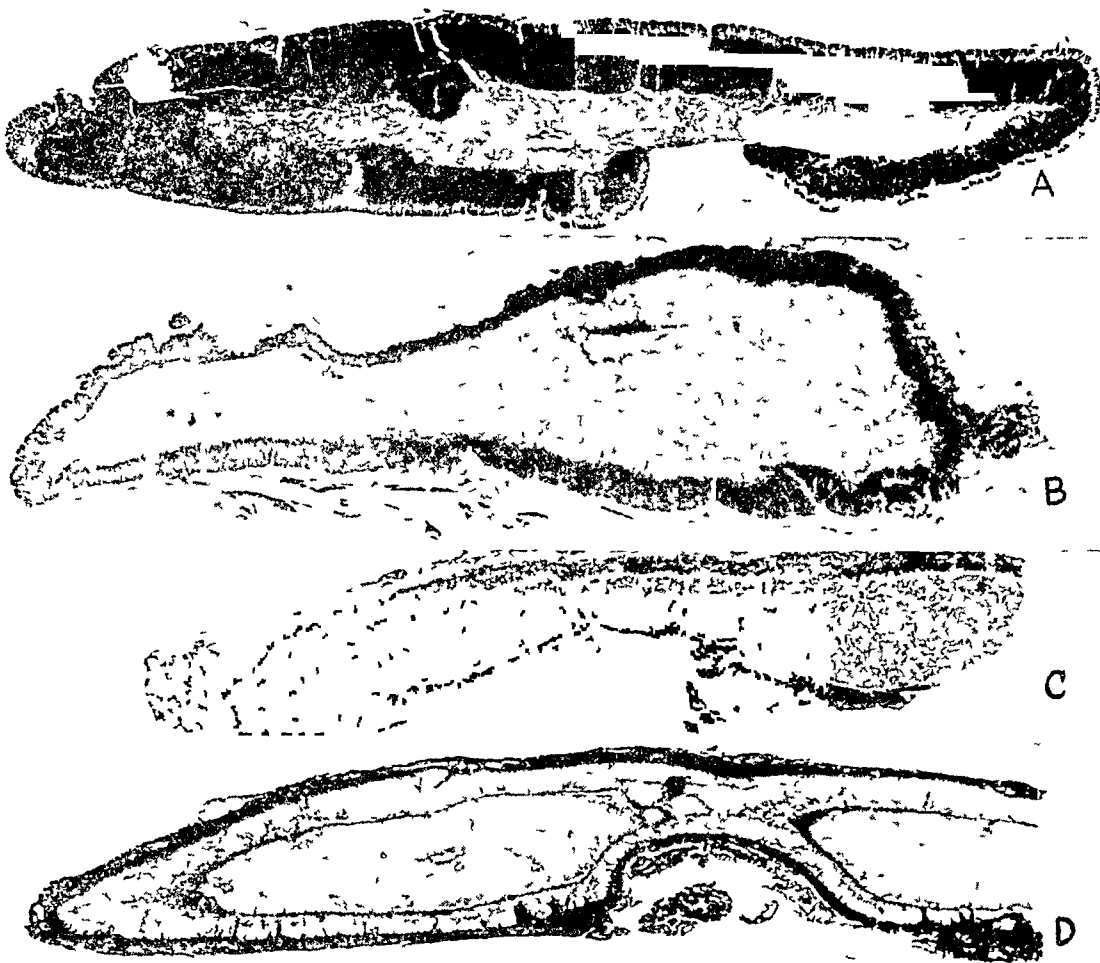


Fig 1—*A*, frozen section of an adrenal gland of a control dog of the same weight as the average of those fed TDE, stained with oil red O and counterstained with hematoxylin, low magnification. The magnification of *B*, *C* and *D* in this figure is greater than that of *A*.

B, frozen section of an adrenal gland of dog M-225, same stain as in *A*. Note the great reduction in the width of the cortex (darker portion).

C, frozen section of an adrenal gland of dog M-201, same stain as in *A*. Note the great reduction in the width of the cortex (darker portion).

D, frozen section of an adrenal gland of dog 82-198, Mallory connective tissue stain. Note the small amount of fibrous tissue between the markedly narrowed cortex and the medulla as compared with the larger amount in the capsule. It is apparent that any cortical fibrosis present is chiefly relative, from loss of parenchyma.

periportal fibrosis, slight proliferation of small bile ducts, slight portal lymphoid cell infiltration and portal macrophages containing small amounts of hemosiderin. Only in the dog surviving for the shortest period, thirty-four days, was there definite necrosis of hepatic cells.

Kidney—The kidney was unaffected by TDE except that the average fat content of the tubular epithelium was about double the normal. In our experience this is a fairly common and nonspecific reaction of the dog kidney to toxic agents. Glomeruli and blood vessels were unaltered, and no "spontaneous" nephritis was present.

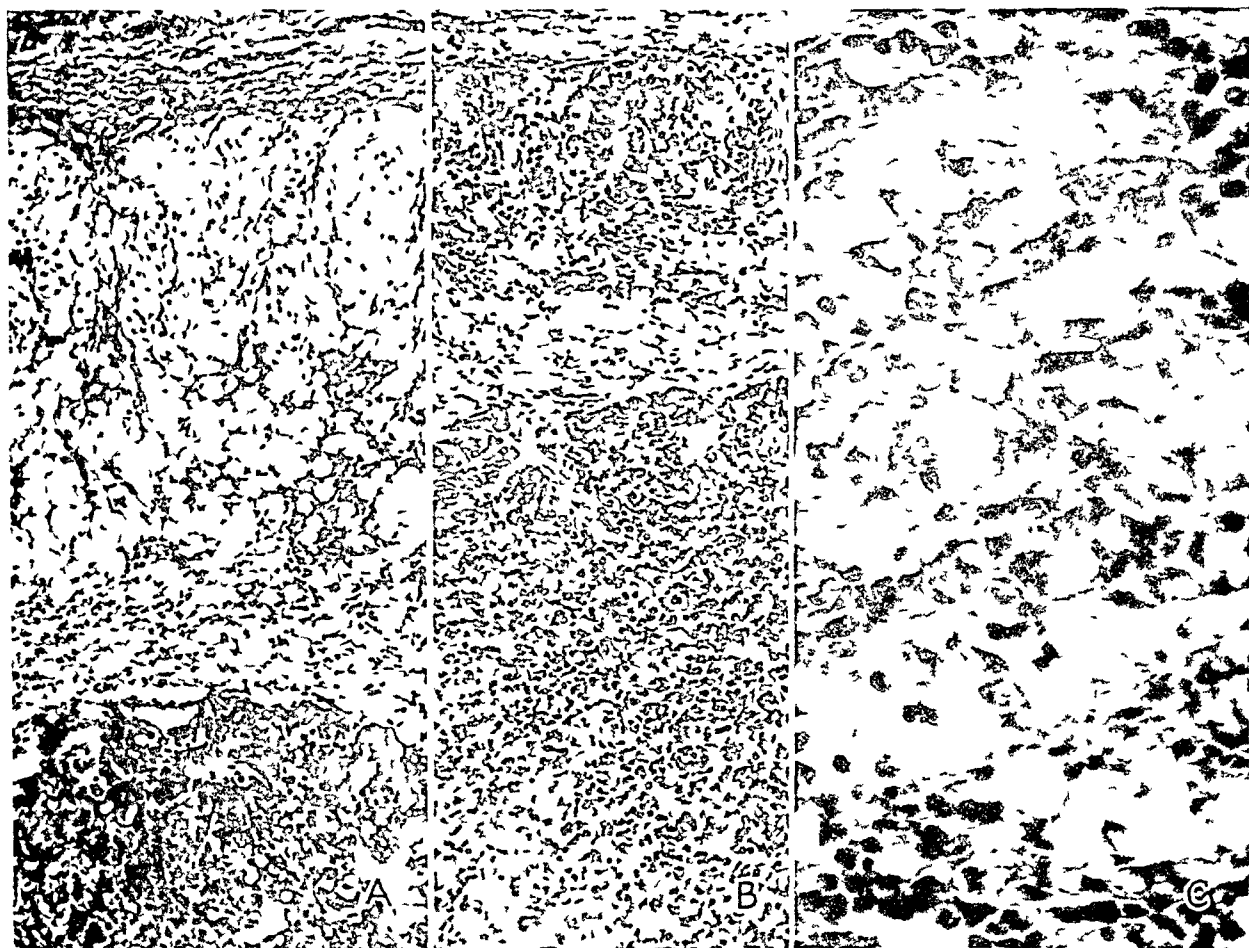


Fig 2—*A*, section of an adrenal gland of dog M-198, hematoxylin-eosin stain, medium magnification. Note the foamy appearance of the remaining cortical cells and the marked narrowing of the cortex. The edge of the medulla occupies the lower third of the print.

B, section of an adrenal gland of dog 82-198, same stain and magnification in *A*. Note the even greater narrowing of the cortex, now only a thin rim. About half the thickness of the medulla is shown below.

C, portion of adrenal cortex of dog 90-219, showing the foamy appearance of the remaining cortical cells, and smaller cells of various types as described in the text, high magnification.

Bone Marrow—The marrow showed slight changes, of the type often seen accompanying death from toxic substances. With some inconsistencies, there were on the average a slight increase in the myeloid-erythroid ratio, a reduction in the number of mature granulocytes and a slight shift to the left in the myeloid line,

the metamyelocytes and myelocytes being increased in number. These changes were judged from inspection of both sections and smears, counts were not made. In at least 2 dogs, it was thought, the erythroid elements were decreased in absolute number.

Incidental Lesions—As mentioned in connection with the gross description, there were a few lesions, each occurring in but 1 dog, which were of uncertain relation to treatment or at least not in causative relation to the adrenal lesions. To save space, only the diagnoses will be given here. Each of the lesions was visible grossly as well as microscopically. The lesions were moderate subacute focal myocarditis in dog M-200, marked erythrophagia of recent appearance in the mesenteric lymph nodes of dog M-198, a small amount of recent mucosal hemorrhage in the neck of the urinary bladder of dog M-228, a moderate amount of recent hemorrhage of the stomach mucosa in dog M-201, focal papillary proliferation of the prostatic epithelium in dog 82-198 and massive pyometra in dog M-202. The total amount of parasitism in this group of dogs was relatively small.

Other Structures—The spleen contained a possibly slight excess of hemosiderin over the small amount normally present. Except for the incidental possibilities mentioned in the previous paragraph, lymph nodes, heart, gallbladder, lungs, pancreas, stomach, intestines, urinary bladder, thyroid and parathyroid glands, ovaries, uterus, testes, prostate, voluntary muscles, brain and hypophysis were not affected by TDE, and in all these structures the microscopic appearance was that seen in normal young adult to middle-aged dogs.

COMMENT

From the chemical point of view the adrenal lesion described is a remarkable example of chemical specificity as related to damage of an organ. From the pathologic point of view the considerable morphologic resemblance to human Addison's disease of the idiopathic or cytotoxic type raises the question of the possible position of various chemicals in the etiology of the human condition. However, we simply state the latter possibility and do not wish to stress it. We do not infer that human beings rather heavily exposed to TDE, e g, pest control operators, are necessarily liable to adrenal damage. In all probability, some people react to a toxicant more in the fashion of a rat than a dog, and other people do the opposite. Since man is now exposed to an ever increasing number of chemicals, it becomes of some importance to determine the similarities and differences of human and animal reactions to these chemicals.

Perhaps first a brief mention of the proper name for the pathologic process seen in these dogs may be appropriate. We have labeled it "severe cortical atrophy" with the alternative designation of "cytotoxic" to agree with the more common name for the similar morphologic picture seen in man. The process is certainly not a simple type of atrophy such as may occur in certain other organs during slow starvation or in the adrenal gland itself following hypophysectomy or treatment with adrenal cortex extracts. Actually, it seems to be a slow necrosis with a certain amount of simultaneous attempt at repair, and the histologic features indicating this have already been stated. Other

names for this type of adrenal lesion of man are given by Weiner³ In a recent and detailed discussion Friedman⁴ used the term "adrenocortical contraction"

Spontaneous disease of the adrenal cortex of a type histologically comparable to certain forms of Addison's disease seen in man apparently does not occur in animals, judging from a reasonably thorough search of the literature Such a condition, on the basis of subtotal vascular occlusion, has been produced by the experimental surgical procedures of Rogoff,⁵ principally in cats We have seen no previous reports of its having been produced in animals on the basis of chronic toxicosis except to a limited degree in guinea pigs by Humphreys and Donaldson,⁶ who used a German preparation of the type of suramin sodium U S P We do recognize that less severe atrophies of a more simple type have been produced on such a basis and that severe acute damage of the adrenal gland has been produced by a variety of methods Implication of chemical toxicants in human Addison's disease seems limited to the aforementioned German proprietary drug (germanin or Bayer 205)⁷

We have done no blood chemical, hematologic, hormonal or metabolic studies on the dogs reported on in this paper

Only 2 of the dogs had been used in previous toxicity experiments Strangely enough, these were the only 2 that showed cirrhosis of the liver grossly and microscopically, the condition was pronounced in dog 82-199 and of lesser degree in dog 82-198 A year and a half previously these 2 dogs had finished an eight months' course of feeding of 100 mg of 3-methyl-4-(4-diethylamino-1-methylbutylamine)-7-chloroquinoline (SN 6911, an antimalarial drug) per kilogram per day, apparently without effect (Two other dogs given the same course of feeding of this drug had shown only minor alterations of the liver on microscopic examination, these dogs, however were mongrels, while the 2 later given the TDE were purebreds)

Bronchopneumonia or other infection as a contributing cause of death was essentially absent, the one exception was dog M-202, which died of pyometra nine days after being mated

The animal toxicity studies of TDE (DDD) reported in the literature include those of Lillie and associates⁸ and Haag and associates⁹ The former studied 8 rabbits treated for periods up to

3 Weiner, H. A. *Am J Path* **12** 411, 1936

4 Friedman, N. B. *Endocrinology* **42** 181, 1948

5 Rogoff, J. M. *Arch Path* **38** 392, 1944

6 Humphreys, E. M., and Donaldson, L. *Am J Path* **17** 767, 1941

7 Wells, H. G., Humphreys, E. M., and Work, E. G. *J A M A* **109** 490, 1937

8 Lillie, R. D., Smith, M. I., and Stohlman, E. F. *Arch Path* **43** 127, 1947

9 Haag, H. B., and others. *Indust Med* **17** 477, 1948

thirty-nine days and stated that "the adrenal glands were regularly normal, with lipid depletion of the glomerular zone in 2 of 7" In 2 rats dying acutely, there was "some fatty degeneration of medulla cells in the adrenal glands" Haag and co-workers included the adrenal glands among the organs studied histologically when they exposed dogs, rabbits and rats to this toxicant by a variety of routes, but reported no lesions in them The dogs had dust atmosphere and spray atmosphere exposure Unpublished studies of rats, mice, rabbits and 2 monkeys of the Division of Pharmacology of the Food and Drug Administration have shown no adrenal damage from TDE

SUMMARY

Ten dogs were studied grossly and microscopically after being fed the insecticide TDE (also called DDD, chemically, 2,2-bis [para-chlorophenyl]-1,1-dichloroethane) at levels of 50 to 200, usually 50 or 80, mg per kilogram per day for periods of one to thirty-three months In every one there was a high grade of adrenal cortical atrophy of a cytotoxic type The adrenal cortex was from one half to one third or less of its usual thickness, and microscopically there was much distortion of the normal structure with alteration of the normal cellular appearances The adrenal medulla showed no changes

Of some dozens of compounds fed to over 300 of our dogs, none except TDE has caused adrenal cortical atrophy, even though several have caused severe hepatic damage, few have affected the adrenal gland in any way, even though they differed chemically from TDE as little as the presence of a single additional chlorine atom in the molecule

In other animal species studied by ourselves and others TDE caused little if any adrenal damage

Males and females, purebred and mongrel dogs, were affected alike In addition to the adrenal gland the liver was uniformly affected, the principal lesion being fatty degeneration The kidneys contained a greater than usual amount of fat Among other structures the hypophysis, the testis or the ovary, the pancreas, the thyroid gland and the parathyroid gland of every one of the 10 dogs were examined, and none of these structures showed any effect attributable to TDE

Morphologically, the condition in the adrenal gland of the dog has considerable resemblance to that observed in the adrenal gland of man in some instances of Addison's disease of the idiopathic or cytotoxic type, but we are not stressing either the morphologic resemblance or any idea of a specific chemical cause of the latter The effect of TDE on the dog adrenal gland is, however, a striking example of chemical specificity in the causation of organic damage

HEPATIC LESIONS PRODUCED BY LEAD IN RATS FED A HIGH-FAT DIET

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AND

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ALL FORMS and degrees of damage of the liver have been described in lead poisoning: fatty infiltration or degeneration, hepatic cell degeneration with nuclear changes, fibrosis with lobular atrophy, and cirrhosis, among others.¹ Nevertheless, authors do not agree on the importance and the constancy of the hepatic injuries produced by lead.

In their book, Cantarow and Trumper^{1b} concluded: "Despite the fact that hepatic cellular damage does not occur consistently in clinical or experimental lead poisoning, there seems little doubt that under certain conditions lead is at least able to contribute to the development of such damage which if protracted may eventuate in cirrhosis of the liver."

In 21 cases of acute lead poisoning in which the patients were children, Blackman² found inclusion bodies in the liver cells, many abnormal nuclei with or without inclusions, and occasional necrotic cells. In the majority of cases there was evidence of destruction of a few periportal liver cells together with slight chronic inflammatory reaction and scarring in each portal area. He also found somewhat similar hepatic lesions in rats given lead carbonate with drinking water.

On the other hand, rats fed a standard diet and poisoned during periods of three weeks to twenty-four months failed to show any conspicuous damage of the liver.³

The occurrence of hepatic lesions in lead-poisoned rats fed a high fat diet induced us to study the subject further. The results are presented in this paper.

From the Instituto de Biología y Medicina Experimental

1 For a review of the literature see (a) Schmidt, P. *Bleivergiftung*, Berlin, Urban & Schwarzenberg, 1930, (b) Cantarow, A., and Trumper, M. *Lead Poisoning*, Baltimore, Williams & Wilkins Company, 1944.

2 Blackman, S. S., Jr. *Bull. Johns Hopkins Hosp.* 58: 384, 1936.

3 (a) Finner, L. L., and Calvery, H. O. *Arch. Path.* 27: 433, 1939. (b) Fairhall, L. T., and Miller, J. W. *Pub. Health Rep.* 56 (pt. 2): 1610, 1941. (c) Chiodi, H. Unpublished Data.

MATERIAL AND METHODS

White rats from our institute strain weighing 150 to 200 Gm were fed twice a day through a rubber tube, no 10 introduced down to the stomach. A 10 cc B-D Sana Lock syringe was attached to the rubber tube and 7 to 15 cc of the diet mixture was given each time, according to the animal's weight.

Lead poisoning was produced by giving daily, by stomach tube, 2 cc of a 4.5 per cent lead acetate solution in distilled water slightly acidified with acetic acid.

Diets—A high fat diet was fed to the rats, made up as follows: corn starch, 29 per cent, wheat flour, 20 per cent, casein, 10 per cent, McCollum's salt mixture,⁴ 1 per cent, cod liver oil, 5 per cent, Mazola corn oil, 35 per cent. The total protein content was 14.7 per cent. To render the diet mixture more fluid, 70 cc. of tap water was added to each 100 Gm of the diet.

The following materials were incorporated in the diet as further specified:

Choline chloride 60 mg per hundred cubic centimeters of the fluid diet, or about 10 to 12 mg per rat, daily.

Inositol Same as choline.

Tocopherols 30 mg of a tocopherols mixture (Parke-Davis) per hundred cubic centimeters of the fluid diet or not less than 3 mg of alpha tocopherol to each rat, daily.

Dried brewers' yeast 5 per cent at the expense of an equivalent amount of corn starch.

Casein 30 per cent instead of 10 per cent at the expense of equivalent amounts of corn starch and wheat flour.

Methionine 500 mg per hundred cubic centimeters of fluid diet, or around 75 to 100 mg per rat, daily.

Cysteine Same as methionine.

A high carbohydrate diet consisted of: corn starch, 44 per cent, wheat flour, 40 per cent, casein, 10 per cent, cod liver oil, 5 per cent, McCollum's salt mixture, 1 per cent. Total protein content, 18.7 per cent.

Determination of Total Lipids and of Hemoglobin—For total lipids the analytic procedure was similar to that employed by Handler⁵—two alcohol-ether extractions of ten minutes each, instead of one of five minutes, being made to insure that all lipids had been extracted. The phosphorus content of lipids was determined by the Fiske-Subbarow method. Phospholipids were calculated by assuming an average of 4 per cent phosphorus content.

Hemoglobin was determined with a Sahli-Hellige hemoglobinometer on blood obtained from the tail.

Microscopic Study—Liver samples taken from the large left lobe were fixed in Zenker's solution as modified by Helly, Bouin-Hollande fluid^{5a} and 4 per cent formaldehyde solution. Paraffin sections were stained with hemalum-eosin and

4 McCollum's salt mixture contains: sodium chloride, 146 Gm, anhydrous magnesium sulfate, 225 Gm, sodium biphosphate (sodium dihydrogen phosphate plus water), 293 Gm, dipotassium hydrogen phosphate, 805 Gm, tetrahydrogen calcium phosphate plus water, 456 Gm, ferric citrate, 100 Gm, calcium lactate, 1,098.5 Gm.

5 Handler, P. J. Biol. Chem. **173** 295, 1948.

5a Bouin-Hollande fixative fluid is made up as follows: copper acetate, 25 Gm, trinitrophenol, 40 Gm, formaldehyde solution (40 per cent), 100 cc, distilled water, 1,000 cc, acetic acid, 10 cc.

with Mallory's connective tissue stain as modified by Heidenham (azocarmine G or B substituted for fuchsin) For reticulin and nuclei the Rio Hortega method was used Frozen sections were stained for fat with sudan III and hematoxylin

RESULTS

Survival, General Condition and Body Weight—The general condition of the rats fed the high fat diet was rather poor, gain of body weight being small or nil Lead administered to these rats caused severe diarrhea, with a very poor general condition resulting, death followed within six to nineteen days (table) At autopsy an enormous dilatation of the stomach was found

Choline added to the high fat diet did not prevent the symptoms or death caused by lead

Rats fed the high fat diet with choline, inositol and yeast and poisoned with lead lived longer than those given lead which did not receive yeast Nevertheless, only 1 rat of the former group survived more than thirty-eight days, as shown in the table The general condition of these animals was good until a few days

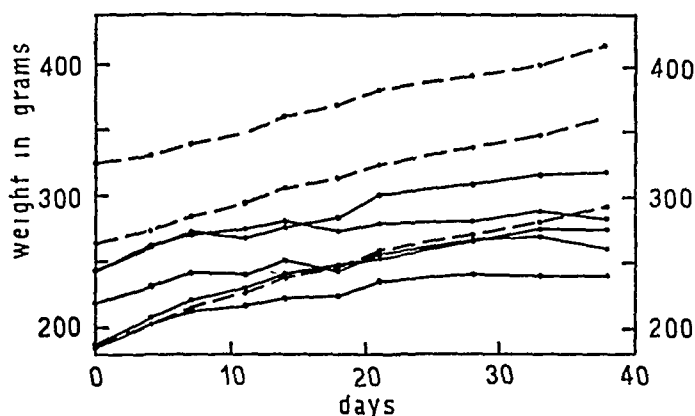


Fig 1—Growth curve of lead-poisoned (—) and control rats (— —) fed the high fat diet with choline, inositol and yeast

before death, at which time diarrhea and gastric dilatation appeared Figure 1 shows that the animals which were given lead and the high fat diet with choline, inositol and yeast gained weight at a slightly lower rate than the controls As between poisoned and control rats, weight differences were more conspicuous in those which did not receive yeast

Controls fed the high fat diet with choline, inositol and yeast were in good condition at the end of the experiment, their weight gain was considerable, as shown in figure 1

When methionine, cystine or 30 per cent of casein was added to the high fat diet with choline, inositol and yeast the whole intoxicated group survived throughout the experimental period The general condition and the weight gain were the same as in the controls If yeast was omitted from the diet, casein did not show any favorable effect on the life span of poisoned rats

Animals fed the high carbohydrate diet, whether poisoned with lead or not, were in good health throughout the whole experimental period

Hemoglobin—Rats fed the high fat diet with choline, inositol and yeast showed a more severe anemia than that observed with a standard diet given ad libitum to

8 rats In the first group (table) extreme values were 32 and 45 per cent (Sahl) and in the latter 58 and 74 per cent, poisoning period and doses being the same

Controls given the high fat diet with choline, inositol and yeast showed normal values (table)

Casein did not modify the degree of the anemia Methionine and cystine seemed to improve anemia slightly but, owing to our scanty experimental material, we could not reach any conclusion

Liver—High Fat Diet The liver was enlarged in both poisoned and control rats, although more in the former, whether absolute weight or percentages of body weight were considered (table) In poisoned animals the liver was darker than normal, the edges being obtuse, but there was no change in the organ surface Fat percentages were high in both groups, although more in controls than in lead-poisoned rats, phospholipids were low, as shown in the table

Histologic examination showed an increase in size of the hepatic cells, with giant nuclei, in all the 9 poisoned rats In 5 instances fatty infiltration or degeneration was present Necrosis around the central vein of the lobule was found in 1 case

In the controls there was fatty infiltration without any nuclear change

High Fat Diet with Choline In regard to liver changes the poisoned rats were rather similar to the preceding group, although fat content was lower (table) The liver of 1 rat weighed 16.2 Gm, the average of the remaining 3 was 10.4 Gm

It must be mentioned that only in 1 of the 4 intoxicated rats was the liver fat as high as 11.2 per cent, in the remaining 3 it ranged from 6.1 to 6.7 per cent Phospholipids amounted to 23.6 per cent in the former and averaged 46.7 per cent in the latter

Among controls given a high fat diet, the group receiving choline showed a clearcut decrease of the liver fat when compared with that not given choline

In the lead-poisoned rats the liver showed giant nuclei as in the group not given choline, fatty lesions being present only in the single animal with a large liver

High Fat Diet with Choline and 30 per Cent Casein A mild fatty infiltration of the liver without any hypertrophy of the nuclei was found in poisoned animals, differences of liver size between control and intoxicated rats were small (table)

High Fat Diet with Choline and B Complex⁶ In the poisoned rats the liver changes were similar to those found in the group fed the high fat diet with choline

High Fat Diet with Choline, Inositol and Tocopherol Microscopic study of 1 of the 3 poisoned rats showed giant nuclei in the hepatic cells without fatty infiltration

High Fat Diet with Choline, Inositol and Yeast Although there were differences of liver size between poisoned rats and controls in this group, they became smaller in the rats which survived fifty-two days

Liver fat and phospholipid percentages were lower in rats given lead which survived thirty-eight days than in controls and only slightly higher than those found in poisoned rats fed the high carbohydrate diet (table) As expected, fat percentages were higher in the animals which lived longer

Histologic examination of the livers of 7 rats of the poisoned group showed marked hypertrophy of the nuclei in 3 of them (fig 2 B to E), less in the remaining ones Mitotic figures were observed in 1 (fig 2 F) There were foci of necrosis around the central vein of the lobule in 4 rats (fig 2 E) and perilobular

6 Dissolved in 15 cc of distilled water the rats were given pyridoxine, 0.06 mg, riboflavine, 0.09 mg, nicotinamide, 0.75 mg, calcium pantothenate, 0.6 mg, thiamine, 0.045 mg, p-aminobenzoic acid, 3 mg

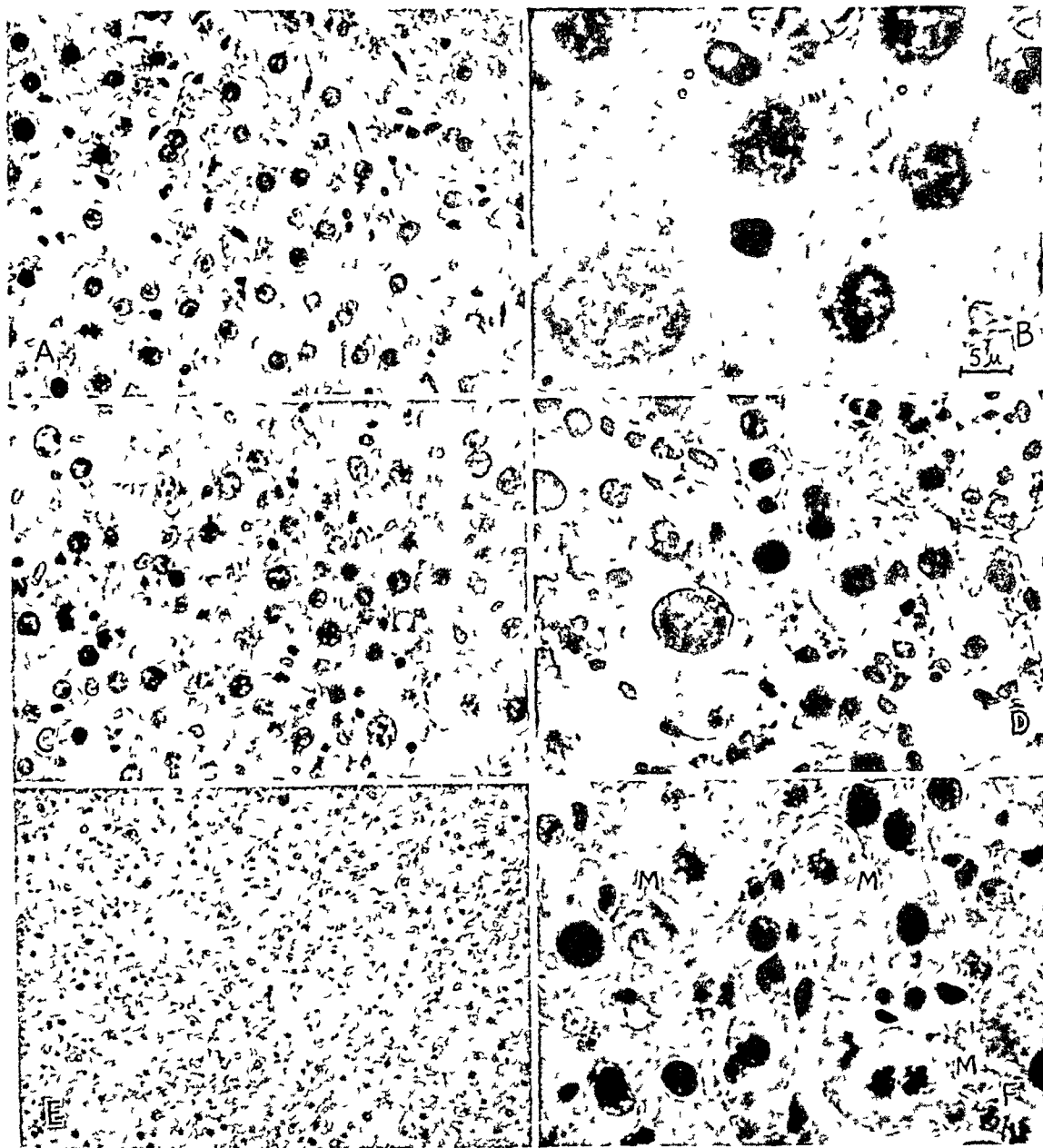


Fig 2—*A*, liver of control rat fed the high fat diet with choline, inositol and yeast during thirty-eight days. Hematoxylin-eosin, $\times 250$

B, higher magnification of part of *C*, showing a giant nucleus. Hematoxylin-eosin, $\times 643$

C, liver of lead-poisoned rat fed the high fat diet with choline, inositol and yeast during thirty-four days. Regeneration of the parenchyma and giant nuclei are shown. Hematoxylin-eosin, $\times 250$

D, hypertrophied cell with a giant nucleus. Silver ammonium carbonate, Hortege's technic, $\times 428$

E, liver of lead-poisoned rat fed the high fat diet with choline, inositol and yeast during thirty-seven days. Centrilobular necrosis and hemorrhage are seen. Note some hypertrophied nuclei. Hematoxylin-eosin, $\times 128$

F, mitosis (*M*) of the hepatic cells of a rat treated with lead and fed the high fat diet with choline, inositol and yeast during twelve days. It was the only case in which mitotic figures were observed. Silver ammonium carbonate, Hortege's technic, $\times 428.5$

Results of Experiments

Lead Poisoned Rats

Microscopic Lesions of Liver														
Liver										Nuclear Hypertrophy				
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* The rats were killed with illuminating gas

† A microscopic study was made of 1 rat only

‡ This diet contained 30 per cent casein instead of 10 per cent

§ Very slight hypertrophy was noted

fibrosis with derangement of the parenchyma and trabeculae in another 4. Slight fatty infiltration was observed in 2 poisoned rats but in none of the controls (fig 2A)

High Fat Diet with Choline, Inositol, Yeast and 30 per Cent Casein The liver weights of the poisoned rats were not increased whether expressed as absolute values or as percentages of the body weight (table)

Nuclear hypertrophy was present in the liver of 2 of the 6 intoxicated animals which were examined. The hepatic parenchyma was normal except in 1 case in which perilobular sclerosis was found.

In controls, hepatic cells were infiltrated with tiny droplets of fat, and periportal fibrosis and proliferation of the bile ducts were present.

High Fat Diet with Yeast, Given ad Libitum Lead-poisoned animals receiving this diet diminished their daily intake of food, lost body weight and died in sixty to seventy-five days. The livers of the 5 poisoned rats showed hypertrophy of the nuclei of the hepatic cells, without fatty infiltration, which was present in 1 of 3 controls receiving the same diet.

High Fat Diet with Choline, Inositol, Yeast and Methionine There was no difference in size of liver between poisoned rats and controls (table). One of the 3 poisoned rats showed slight nuclear hypertrophy, and small foci of necrosis of the central part of the lobule were present in all. In controls the hepatic parenchyma was normal.

High Fat Diet with Choline, Inositol, Yeast and Cystine There were small foci of necrosis around the central vein of the lobule, edema and hemorrhagic foci in both control and poisoned rats. In the latter there was also mild nuclear hypertrophy.

High Carbohydrate Diet, Low in Fat The livers of lead-poisoned rats were slightly heavier than those of controls. The fat and phospholipid percentages can be considered within normal limits for a diet rather low in choline and without yeast (table).

In 1 of the 5 poisoned animals there was slight hypertrophy of hepatic nuclei, and glycogen infiltration of the parenchyma was present in 3 of them.

Nuclear Lesions—There was hypertrophy of the nuclei of the hepatic cells, mostly located around the center of the lobule, the cells being also enlarged. There was a loss of the characteristic radial arrangement of the columns of hepatic cells. Diameters of the hypertrophied nuclei were quite outside normal limits (upper limit, 15 to 16 microns instead of 6 to 8 microns, as shown in fig 2B). Giant nuclei showed the chromatin irregularly distributed in granules or in a thick net (prophase), one, two or sometimes more acidophilic nucleoli being present. In some cases one or more vacuoles filled the nucleus almost completely. There were also cells which were devoid of nuclei. Binucleated cells were present in larger number in lead-poisoned livers than in those of controls.

It must be noted that in none of the examined livers were clear macroscopic signs of fibrosis or necrosis found.

COMMENT

When lead acetate was given to rats forcedly fed a high fat diet, severe hepatic damage and anemia developed consistently, and the animals died within three to eighteen days. The addition of choline or inositol to the high fat diet did not lengthen the time of survival. When yeast was included in the diet, rats survived longer, though only by exception more than thirty-eight days.

If the high fat diet with yeast was fed ad libitum to the poisoned rats, there was a diminution in their daily intake of food, which resulted in a slow process of undernutrition and finally in death after sixty to seventy-five days

By increasing to 30 per cent the casein content of the high fat diet with choline, inositol and yeast, the death of poisoned animals was prevented within an experimental period of thirty-four days. If yeast was omitted, casein had no effect on the time of survival.

That death of rats receiving lead was caused by overfeeding of fat is shown by the fact that a high carbohydrate diet low in fat allowed poisoned rats to survive in good health for at least forty-six days. Likewise, rats kept on a standard diet and receiving similar doses of lead remained alive for one year.^{6a}

Increase in the size of the liver in the rats receiving lead and a high fat diet was independent, partially at least, of the fatty infiltration of the organ, since it was observed in cases in which the latter was not present.

It might be thought that the hepatic lesions could be due only to a high fat content or a protein deficiency of the diet, but the fact that such lesions were not present in the controls fed the same diet allows us to discard such an assumption. A further proof is given by the observation that the lesions characteristic of lead poisoning were present even in those cases in which fatty infiltrations or degeneration was completely prevented by lipotropic agents and yeast or tocopherol.

Increasing the protein content of the diet to 30 per cent prevented hepatic damage from lead in most of the rats. Methionine was somewhat less active, and cystine almost inactive. Yeast was not necessary for the preventive action of casein but was necessary for the prevention of death of the lead-poisoned rats.

Lead acetate given without an excess of fat did not produce conspicuous hepatic damage, as found in the rats fed a high carbohydrate diet low in fat or in those fed a standard diet ad libitum and poisoned daily during three months with 2 cc of 12 per cent lead acetate solution.^{7c} The lack of damage of the liver may be explained by a low hepatic storage of lead⁷, administration of fat would increase the lead storage, as found in rabbits by Weyrauch and Necke.⁸ The damage caused by lead is thus very similar to that caused by trinitrotoluene, which produces injuries of the liver only when the rats are restricted to a high fat diet.⁹

6a Chiodi, H., and Sammartino, R. A. *Nature*, London **160** 680, 1947

7 Laug, E. P., and Harris, H. P. *J. Pharmacol. & Exper. Therap.* **64** 388, 1938

8 Weyrauch, F., and Necke, A. *Ztschr. f. Hyg. u. Infektionskr.* **114** 629, 1933

9 Himsworth, H. P., and Glynn, L. E. *Clin. Sc.* **4** 421, 1939-1942

The increased waste of proteins occurring in lead poisoning,¹⁰ particularly of sulfur-containing proteins, perhaps exaggerated by fat over-feeding, could produce a protein deficiency, which in turn would be the origin of the hepatic damage described in our rats. The preventive action of casein and methionine speaks in favor of such a hypothesis.

According to Hammet,¹¹ quoted by Cantarow and Trumper,^{1b} the retardation of the growth rate of roots and chick embryos produced by lead is due to suppression of cell proliferation, mitotic nuclei apparently showing the greatest affinity for the toxic elements, which was also found in the nuclei and walls of other cells. This author concludes that lead enters into combination with an organic sulfhydryl compound analogous to glutathione, if not this compound itself. However, Vannucci¹² found an increase of the reduced glutathione in the liver of lead-poisoned guinea pigs.

Later studies confirmed the toxic effect of lead on mitosis.¹³

Another thing in favor of the belief that lead exerts a specific action on cellular differentiation and division is the fact that giant nuclei are present in the cells of the renal tubules of the rats^{3c} and the nuclear changes occur in the erythropoietic cells of the bone marrow of the dogs^{3c} poisoned with lead.

Therefore, lead would retard or inhibit, partially at least, the mitosis of hepatic cells, which, being unable to undergo division, would continue to grow, becoming very large or even giant cells. This abnormal cellular growth would explain the increase in the weight of the organ found by us. A similar mechanism has been made responsible for the increase of the size of the kidneys in lead poisoning.^{3a}

In favor of the mitosis-inhibiting action of lead stands the fact that except in 1 case mitosis was rarely seen even in livers which showed the utmost nuclear hypertrophy. Dawson's statement¹⁴ that changes in the size and the number of the nuclei of mammals' liver cells would arise from a variable degree of failure of mitosis points in the same direction.

In brief, it appears that when rats fed a high fat diet are poisoned with lead, there is an abnormal inhibition or waste of sulfhydryl-containing proteins of the hepatic cells, which disturbs the enzymatic processes of mitosis. When the toxic injury is not severe, it retards or partially inhibits the mitosis of a number of cells, which continue to grow until

10 Terroine, T. *Trav Soc chim biol* **26** 1179, 1944.

11 Hammet, F. S. *Protoplasma* **4** 187, 1928, **5** 135, 187, 535 and 547, 1929, cited by Cantarow and Trumper^{1b}.

12 Vannucci, F. *Giord clin med* **14** 585, 1933.

13 Levan, A. *Nature, London* **156** 751, 1945. Mangenot, G., and Carpenter, S. *Compt Rend Soc de biol* **139** 268, 1945.

14 Dawson, A. B. *Anat Rec* **102** 393, 1948.

the inhibition is overcome or until the too large size of the cells interferes with the metabolic process. In other cells the toxic action, being too severe, causes cell death, producing foci of necrosis and afterward fibrosis if the animal survives long enough. Casein and methionine compensate the losses of sulfhydryl-containing proteins caused by lead and prevent the toxic injuries of the liver.

It is worth mentioning the recent work of Orr and Price,¹⁵ who found that para-dimethylaminoazobenzene, a carcinogenetic agent, when given to rats in large doses produces hepatic lesions similar to those described in this paper.

SUMMARY AND CONCLUSIONS

Rats forcedly fed a diet containing 40 per cent fat were given daily 2 cc of a 4.5 per cent lead acetate solution. Death followed within six to nineteen days and the livers showed cellular and nuclear hypertrophy, fatty infiltration or degeneration and necrosis. Addition of choline, inositol, tocopherol and brewers' yeast prevented fatty infiltration or degeneration in most of the animals, but not cellular and nuclear hypertrophy and necrosis.

Casein and, to a smaller degree, methionine prevented cellular and nuclear hypertrophy and necrosis of the liver when added to the high fat diet with lipotropic agents in the presence or the absence of yeast.

Lead acetate given without an excess of fat did not produce conspicuous hepatic damage or the death of the rats within the experimental period.

Casein prevented the death of the animals poisoned with lead and fed the high fat diet within the experimental period only when yeast was present in the diet.

Possible mechanisms of the nuclear changes are discussed.

¹⁵ Orr, J. W., and Price, D. E. *J. Path. & Bact.* **60**: 461, 1948.

CARDIAC HYPERTROPHY IN EXPERIMENTAL ARTERIOVENOUS FISTULA

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AND

HUGH BRAWNER

DURHAM, N C

THE PATHOPHYSIOLOGIC and the clinical picture of arterio-venous fistula have been extensively studied ¹ This report is devoted to studies of the effects of experimental arteriovenous fistula on canine heart muscle Interest is centered about three problems which may be stated as follows 1 To what degree does the heart hypertrophy? 2 Does the hypertrophied heart return to normal after elimination of the overload (excision of fistula)? 3 Does an arteriovenous fistula of short duration (five to seven days) produce cardiac hypertrophy?

EXPERIMENTAL PROCEDURE

A fistula was established between the common carotid artery and the external jugular vein in 11 large dogs (11 to 16 Kg) The incisions in the vessels were 2.5 to 3.5 cm in length, and the edges were sutured with fine silk (atraumatic eye suture size 6-0) The suture technic of Deterling ² was used, and emphasis was laid on careful stripping of the adventitia to prevent clotting For the same reason, the intimal surface of the fistula was coated with a few drops of sterile liquid petrolatum U S P (Carrel ³) All the fistulas remained patent, and thrombosis was never encountered despite the fact that anticoagulants were not used The arteriovenous shunt was made as large as possible in order to produce, it was hoped, a pronounced and rapid effect on the heart muscle The choice of the vessels of the neck was indicated by their size and accessibility Whether the effect of an arteriovenous fistula of these vessels may be influenced by the nearby carotid sinus is unknown

The effect on heart muscle was measured by weighing the heart according to the method of Herrmann ⁴ The weight of the cleaned and fixed left and right ventricles devoid of fat, pericardium, vessels and valvular ring was deter-

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1 (a) Gauer, O, and Linder, F Klin Wchnschr **26** 1, 1948 (b) Holman, E Arteriovenous Aneurysm, New York, The Macmillan Company, 1937, (c) Surgery **8** 362, 1940, (d) Ann Surg **112** 840, 1940, (e) **124** 920, 1946

2 Deterling, R A Surgery **18** 679, 1945

3 Carrel, A Bull Johns Hopkins Hosp **18** 18, 1907

4 Herimann, G R Am Heart J **1** 213, 1925-1926

mined Reference is made throughout this paper to the ratio of the weight of the cleaned and fixed ventricles $\times 100$ to the body weight (VW/BW ratio)

Changes in the size of the heart were followed by lateral roentgenograms of the chest taken at a distance of 91.5 cm (36 inches)

RESULTS

Effect of a Fistula of Long Standing—The first series comprises 3 dogs in which fistulas were established, respectively, for 87, 88 and 93 days Their VW/BW ratios are 0.811, 0.698 and 0.872

Effect of a Fistula of Short Duration—Eyster⁵ rejected the concept of physiologic work hypertrophy and suggested that cardiac enlargement is a form of injury hypertrophy, brought about by abnormal stretching of the muscle fibers in the initial period of overload (foci of hydropic degeneration, necrosis, etc.) In one series of dogs he constricted the aorta ascendens for a period of 3 to 6 days

TABLE 1—*Synopsis of the Experimental Data*

Dog	Final Weight, Kg	Lived with Fistula, Days	Lived after Excision of Fistula, Days	Weight of Cleaned + Fixed Ventricles, Gm	C + F V W $\times 100$
					B W
Series 1					
a	10.43	93		91.0	0.872
b	12.70	87		103.0	0.811
c	14.52	88		101.3	0.698
Series 2					
d	14.17	5	105	82.8	0.584
e	12.93	5	107	77.5	0.599
f	14.06	7	104	82.6	0.587
Series 3					
g	12.84	77	112	79.1	0.616
h	17.12	72	69	104.1	0.608
i	13.72	80	62	105.0	0.765
k	12.36	67	70	80.7	0.652
l	14.62	73	67	99.5	0.680

To another series he gave massive transfusions, rapidly increasing the total blood volume up to 175 to 200 per cent Cardiac hypertrophy, determined by roentgenologic observation and terminal heart weight, developed in these dogs Eyster concluded that the most important factor leading to cardiac hypertrophy is not increased work of the muscle per se but injury of the muscle and the reaction to that injury

Paralleling his work, we established arteriovenous fistulas in the necks of 3 dogs and excised them after 5, 5 and 7 days The dogs were killed 105, 107 and 104 days after the excision and showed VW/BW ratios of 0.584, 0.599 and 0.587, respectively

Effect of the Removal of a Fistula of Long Duration—The third series comprises 5 dogs who had arteriovenous fistulas for 67, 72, 73, 77 and 80 days and were kept alive for 70, 69, 67, 112 and 62 days after the excision of the fistulas Their VW/BW ratios are 0.652, 0.608, 0.680, 0.616 and 0.765

⁵ Eyster, J. A. E. (a) *Tr. A. Am. Physicians* 42: 15, 1927, (b) *J. A. M. A.* 91: 1881, 1928

COMMENT

Our findings have been compared with the "standard" values determined by Herrmann⁴ on 200 apparently healthy, normal dogs. These, however, were not examined for hypertension or renal disease. Herrmann's table reveals an increasing range of variation of ratios with increasing body weight. This distribution is probably in part due to the somatic differences of the various breeds making up the total. (Rabbits show more constant ratios⁶.)

The results of our roentgenologic studies could not be correlated with the heart weights, and it appears that our method is inadequate.

In contrast to our series 1, Drury and Wightman⁷ succeeded in producing cardiac hypertrophy within 3 months (increase in weight of from 45 to 125 per cent). This was accomplished in rabbits by means of carotid-jugular fistulas. As may be seen in the chart, 2 dogs of series 1 showed VW/BW ratios at the upper limit of normal, one of the ratios was slightly above the average. None exceeded the range of normal established by the 200 dogs of Herrmann. This holds true regardless of what body weight we choose. Some of our experimental animals lost weight, in part due to depressive anorexia. The final live weight was used, the ratios are thus weighted in some of the dogs in the direction of hypertrophy. This part of the experiment permits no positive conclusions. The following suggestions and interpretations are, however, drawn from our experience.

1 The arteriovenous shunts either were not large enough for the size of the animals or did not function long enough to produce clearcut hypertrophy, or both.

2 Dogs may compensate the additional circulatory load more efficiently than rabbits.⁸

The results noted in series 2 do not confirm Eyster's^{5b} work. The VW/BW ratios are slightly below average and well within normal limits, indicating complete absence of hypertrophy (see chart). This, however, does not necessarily invalidate Eyster's work, since our experimental method may not produce sufficient overwork to result in cardiac hypertrophy. For example, if Eyster's findings are correct, our inability to duplicate his results may be due to too small a fistula, resulting in too gradual an increase in blood volume and cardiac output. Since, according to Eyster, the time factor is of prime importance, a small

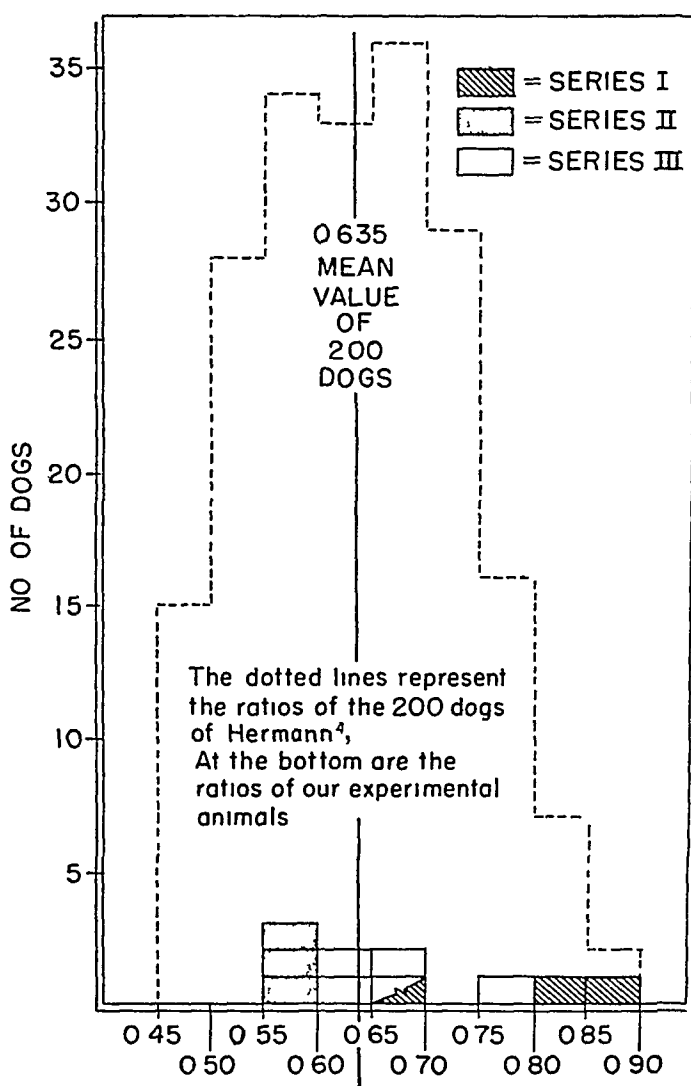
6 Joseph, D. R. *J. Exper. Med.* **10**: 521, 1908. Eyster^{5b}

7 Drury, A. N., and Wightman, K. J. R. *Quart. J. Exper. Physiol.* **30**: 45, 1940. Drury, A. N. *ibid.* **33**: 107, 1945.

8 Ghetti, L. *Chirurg.* **2**: 20, 1947, abstracted, *Internat. Abstr. Surg.* **86**: 59, 1948. Drury and Wightman.⁷

arteriovenous fistula could not effect injury of the myocardial fibers. It would appear that a fair test of his results could be achieved by the production of an aorta-vena cava fistula of a size and a site which barely permit the dog to survive the procedure^{1d}

Dog 1 of the third series has a rather high VW/BW ratio (This animal suffered from pronounced depressive anorexia, and the ratio in



Ratios of weight of cleaned and fixed ventricles $\times 100$ to body weight, compared with Herrmann's⁴ range of normal ratios

our opinion reflects marked loss of weight rather than hypertrophy) With this exception (dog 1) the ratios are fairly well grouped about the average value, indicating possibly a return to normal of slightly hypertrophied hearts (This course of events has been conclusively demonstrated in rabbits⁴) The heart of dog h had 6 adult heart worms

(*Dirofilaria immitis*) floating in the ventricles. Their effect on the cardiac function was apparently nil, since the VW/BW ratio is close to the average value. This dog showed a slight gain in weight.

SUMMARY

An arteriovenous fistula was established between the large cervical vessels in dogs for studying the possibility that the hypertrophied heart may return to normal size after reestablishment of the normal circulation. In dogs with this type of fistula which were maintained for 3 months and then killed, no significant hypertrophy of the heart was found. The hearts of dogs in which the fistula was allowed to remain for $2\frac{1}{2}$ months and which survived its excision for an additional 2 months did not vary in weight from the normal.

VENOUS ATHEROMA

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ABNORMAL thickenings of the venous wall, although not as intensively studied as the corresponding lesions of arteries, have received some sporadic attention. As in the case of "arteriosclerosis," the term "phlebosclerosis" has come to cover a multitude of lesions. The thickenings may be focal or diffuse, they may involve one or two or, more often, all three coats of the vein and they may consist of pathologic material or merely of hyperplastic normal constituents of the vessel.

The condition called venofibrosis¹ is a bilateral symmetric thickening without dilatation of superficial and deep veins due to extensive fibrosis of the media with some irregular thickening of the intima. No calcium or fat was ever seen in these lesions.

Another type of venous thickening has been observed in the portal veins and inferior vena cava in cases of hepatic cirrhosis and congestive cardiac failure². It consists in intimal hyperplasia and muscle appearing in the subendothelial layer, medial hypertrophy of the portal veins and adventitial hypertrophy of the inferior vena cava. Some of the hypertrophied muscle is replaced at a later stage by fibrous tissue. Fat has not been demonstrated.

The increased venous pressure of congestive cardiac failure causes similar adaptive, and later degenerative, change in the hepatic veins³ and in the superior vena cava⁴. In these cases, also, fat was never found in the lesions.

A considerable literature has sprung up about the changes found in the splenic vein in Banti's syndrome and in the portal vein and its tributaries in hepatic cirrhosis, but only in a single case has fatty intimal change been observed.

The same applies to the pulmonary veins. While phlebosclerosis is not uncommon in these vessels in cases of mitral stenosis,⁵ there is

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1 Hauswirth, L., and Eisenberg, A. A. Arch Path **11** 857, 1931

2 Pei-Lin, Li. J Path & Bact **50** 121, 1940

3 Gross, H. Arch Path **23** 457, 1937

4 Gross, H., and Handler, B. J. Arch Path **28** 22, 1939

5 Ljungdahl, M. Untersuchungen ueber die Arteriosklerose des kleinen Kreislaufs, Wiesbaden, F. G. Bergmann, 1915

again only 1 isolated instance in literature of fatty degeneration of the pulmonary vein⁶

If a vein is placed under increased tension by the formation of an arteriovenous fistula, very similar changes occur "The wall of the involved vein becomes thickened and hypertrophied. In addition there is usually a very marked deposition of calcium in the rim of the fistula"⁷

Equally, if a venous segment is transplanted into the arterial circulation great hyperplasia of all coats takes place, leading sometimes to obliteration of the lumen. At other times irregular intimal thickenings have been described⁸. This subject was reported on by Carrel⁹ on various occasions, but neither he nor other observers ever found any fat in the intimal thickenings.

Finally one might mention that Tedeschi¹⁰ observed local fibromuscular thickening in the walls of veins adjacent to arteries, rendering the adjacent side of the vein twice as thick as the opposite side. The thickening was largely due to adventitial hyperplasia.

In conclusion it can be stated that every chronic increase of venous pressure leads to thickenings of the veins involved and finally to degenerative changes. Another striking fact which a survey of the literature brings out is the monotonous unanimity with which authors repeat the statement that they were unable to find fat in the phlebosclerotic lesions. These statements need not always be taken at their face value, for in many papers it is apparent from the description of the technic employed that no attempt was made to find fat (e g, Watts⁸ and Gross³). On the other hand, there is no doubt that most of these lesions do not in fact contain fat, so that reports referring to fatty degeneration of the intima of veins can be counted on the fingers of one hand. Orth¹¹ seems to have seen it in the pulmonary veins in mitral stenosis. Benda expressed himself on the subject thus "I must confess, that I have looked for these intimal fatty changes in vain, the only specimen which I have seen appears to me to be isolated in the literature". He went on, however, to mention intimal fatty change of varicose veins and of the portal vein and reproduced a picture of atherosclerosis occurring in a pial vein of a spinal angioma⁶. Fat is, of course, quite frequently found in organized mural thrombi.

6 Benda, C, in Henke, F, and Lubarsch, O. *Handbuch der Speziellen Pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol 2

7 Reid, M R. *Am J Surg* **14** 17, 1931

8 Watts, S H. *Bull Johns Hopkins Hosp* **18** 153, 1907

9 Carrel, A, and Guthrie, C C. *Compt rend Soc de biol* **59** 412 and 596, 1905, **60** 529, 1906. Carrel, A. *Bull Johns Hopkins Hosp* **18** 12, 1907

10 Tedeschi, C. *Anat Rec* **79** 243, 1941

11 Orth, cited by Benda⁶

It can be said therefore that while pathologic thickening of veins is a well recognized phenomenon, venous atheroma, i e, fatty infiltration of the intima of veins with subsequent fibrosis, hyalinization or calcification, remained a pathologic curiosity up to 1926 when Schilling¹² published his analysis of a lesion which occurs at the origin of the inferior vena cava and which had been noticed by Cramer¹³ in 1921. This lesion Schilling encountered in 50 per cent of all postmortem examinations, and his histologic study of 100 of these plaques revealed fat either alone or associated with hyaline material or calcium in 49 of them. In every other respect the lesions resembled arterial atheroma. Schilling's paper should have received more attention than it did. If true atherosclerosis occurs with great regularity in a certain fixed location in the venous system, this fact may throw light on the causes of atheroma. The purpose of this paper is to add to Schilling's findings and to stress the significance of his discovery.

MATERIAL

In the course of an investigation of atheroma aortae,¹⁴ yellowish plaques were noted to occur with considerable frequency about the origin of the inferior vena cava. It was decided to study the nature of these lesions. In this paper is presented an analysis of 245 cases. They represent an almost unbroken series of all the autopsies at the Royal Infirmary Edinburgh from the beginning of December 1947 to the end of May 1948. There has been no selection other than that introduced by the nature of the hospital, viz, that there are few patients below the age of 12 and few patients with contagious disease. In the majority of instances the inferior vena cava was available only from the renal level downward, i e, about 2 inches (5 cm) of it. In a few cases only the first inch of the vessel was obtained, in other cases the whole subdiaphragmatic length of the vessel could be secured. Together with this lower part of the inferior vena cava were removed in each case the two common iliac veins and the abdominal portion of the aorta with the iliac arteries, to which the veins remained attached. It was found advisable to remove the veins unopened and subsequently to open them from the back, as thereby a better idea of the topography was obtained. The necessity of severing the right common iliac artery in order to open the left common iliac vein was thereby obviated. The specimens were fixed in 4 per cent formaldehyde solution.

The lesions consisted of white or yellow plaques up to 0.5 cm square or streaks up to 2.5 cm in length. In some instances a fairly large area, instead of showing these smooth lesions, was roughened and exhibited a yellowish granularity covering the smooth intima. The plaques were slightly raised and looked much like the nodules of mild arterial atheroma. They occurred in a strictly limited area of the

12 Schilling, W. *Virchows Arch f path anat* **262** 658, 1926

13 Cramer, H. *Virchows Arch f path Anat* **130** 46, 1921

14 Geiringer, E. *Brit J Soc Med* **2** 132, 1948

venous surface which was available for inspection, viz, at and about the orifice by which the left common iliac vein joins the inferior vena cava and in a 2 cm upward prolongation of the line of this orifice (figure)

The plaque-bearing area lay in that part of the vein which is situated entirely behind the aorta and the right common iliac artery. In addition to the proximal end of the left common iliac vein it comprised the lowest 2 cm of the inferior vena cava, which is the only part



Abdominal aorta, lower third of inferior vena cava and iliac vessels. The veins have been opened from the back to display the venous plaque in its typical location in the center of the anterior part of the ostium by which the left common iliac vein joins the inferior vena cava. RCIV indicates the right common iliac vein.

of this vessel which lies entirely behind the aorta, to which it is fixed in this situation. Posteriorly the plaque-bearing area was in relation with the prevertebral fascia, which in this region is usually thin and closely adapted to the front of the lower lumbar region of the spine. For practical purposes the plaque-bearing area of the inferior vena cava and iliac vein is that portion of these vessels which lies between the last lumbar vertebrae at the back and the bifurcation of the aorta.

in front In no other part of the venous surface under investigation were such plaques ever found In 8 instances gross calcification of the plaques was noted In no instance was it possible to squeeze atheromatous material from the lesions, nor was there any macroscopic evidence of ulceration Of peculiar interest is a case in which there was sinistroposition of the inferior vena cava On examination of the venous bifurcation a patch of sclerosis was found in the region of the orifice of the *right* common iliac vein, i e, where in this instance the left common iliac artery crossed the vessel

These findings are in good agreement with those of Schilling, who said that the lesion is usually found "in the middle part of the posterior caval wall shortly above the confluence"¹⁵ The illustration which accompanies Schilling's paper makes it, moreover, certain that he was describing the same lesions

HISTOLOGIC ASPECTS

Frozen sections were prepared from 11 specimens showing the lesion, as well as from some normal-looking veins and from some veins showing the diffuse thickening which is so constant a feature in cases of chronic congestive cardiac failure The sections were stained with scarlet red or sudan III and variously counterstained

The normal inferior vena cava does not possess a subendothelial layer In both the arteries and the veins of the newborn the endothelium comes to lie directly on the internal elastic lamina Connective tissue intervening between these two layers, is soon formed in the larger arteries, but the veins throughout life remain normally devoid of such a subendothelial layer In a normal vein the very soil on which atheroma grows is missing However, in the course of life in many people the inferior vena cava acquires such a subendothelial layer, and this acquisition is constantly found in persons who exhibit increased venous pressure from whatever cause¹⁶

This fact, viz, that the subendothelial layer in which the sclerotic plaque occurs, is not found in normal veins, raises the question whether the plaque is secondarily implanted on an already hypertrophied intima or whether the formation of the subendothelial layer proceeds *pari passu* with the formation of the plaque In this series both types of occur-

15 A slight misconception has crept into this, probably due to the fact that Schilling opened his veins completely before looking at the lesions In the completely opened veins the upper part of the orifice of the left common iliac vein appears as part of the inferior vena cava and one is tempted to call inferior vena cava what is in reality still left common iliac vein The most common and very often the only place at which these plaques are found is in fact the orifice of the left common iliac vein

16 Dijkstra, O H Nederl tijdschr v geneesk 76 2423, 1932 Pei-Lin²

rence were noted. In some cases the plaque occurred in a vein already diffusely hypertrophied and possessing a subendothelial layer, in other cases one could see that the vein above and below the lesion was normal, i. e., devoid of such a layer. In the latter cases it must be assumed that the local subendothelial thickening and the atheroma formation are part of the same process. Indeed, in 5 of the 11 cases the plaque was entirely composed of fibrous elastic and muscular tissue, so that it represented no more than an exaggerated and strictly localized diffuse intimal hyperplasia of the type met with in cases of increased venous pressure. The media in these cases was either normal or atrophied, while the adventitia was usually considerably hypertrophied. These findings correspond with those in the aforementioned diffuse thickenings of the inferior vena cava. The amount of smooth muscle is variable in these connective tissue plaques, and in 2 instances there was evidence of hyalinization. The elastica was often split, with the plaque occurring between the reduplicated layers. This is stressed by Jores¹⁷ as a feature of true arteriosclerosis. Of the 100 cases of Schilling, 30 showed this purely connective tissue composition.

The main question, however, was whether it would be possible to confirm Schilling's results respecting the fat content of these lesions. He had found fat in 49 of 100 cases, either alone or in combination with hyalinization, calcification or both, in 5 of our 11 cases the lesions were found to contain fat. The fat was seen in small droplets finely distributed between the connective tissue cells and fibers. It tended to occur toward the center of the plaque or in the depth toward the media, and in 1 case it was seen to penetrate for a short distance between the muscle cells of the media. Gross conglomerations of fat, cholesterol crystals or typical "foam cells" were not encountered, but small connective tissue cells containing granular fat were numerous in some sections. In 1 case there was partial calcification of the plaque, the picture being similar to that of calcified foci in atheromatous plaques in small arteries. The central core of calcium was surrounded by many small extracellular granules of calcium and droplets of fat suggesting that a calcific metamorphosis of a lipid focus had taken place. In another case small groups of round cells occupied tissue spaces here and there in all three layers of the vessel. Apart from this there was not in any of the cases an appearance suggestive of inflammation.

All these findings agree well with the results of Schilling and fully confirm his most significant discovery that almost half of these intimal thickenings contain intracellular and extracellular fat. In all other essential respects, also, these lesions agree with atheroma, being differ-

17 Jores, L. *Wesen und Entwicklung der Arteriosklerose*, Wiesbaden, J. F. Bergmann, 1903.

ent from arterial atheroma in degree only. While in arterial lesions fat may predominate over connective tissue, venous atheroma shows the inverse relation. While the fat infiltrations of arterial atheroma tend to coalesce, the fat droplets of the venous intima remain discrete, although in 1 specimen some coalescence can be observed. Splitting of the elastica is a prominent feature in both types of lesion. One may thus regard these venous lesions as mild atheroma with a marked tendency to heal by fibrosis.

In conclusion I wish to draw attention to the unbroken series of histologic pictures leading from the normal inferior vena cava to the atherosclerotic one, a series which might point to the pathogenesis of atheroma.

- (1) the normal vein—no subendothelial layer
- (2) the vein under increased venous pressure—formation of a fibroelasticomuscular subendothelial layer
- (3) the connective tissue plaque—a focal and exaggerated replica of 2
- (4) the fibrolipoid plaque—atheroma

As Cramer¹³ said: "Sclerosis of vascular walls is seen in its purest and simplest form in the veins where degenerative changes remain minimal and where, owing to the scanty development of the lesions, we are most likely to gain an understanding of their origin."

INCIDENCE

Of the total number of 245 veins examined, 108, i. e., 44 per cent, showed sclerotic plaques. This is a slightly lower incidence than Schilling's, who found them in 50 per cent. Among the 147 males the incidence was 44.2 per cent, and among the 98 females it was 43.9 per cent. There is thus no sex difference. Neither was there in Schilling's series. The incidence in the various age groups is given in table 1. It can be seen that while there is a definite trend toward more frequent occurrence with advancing age, the correlation is by no means close. In an attempt to uncover etiologic associations the following groups were analyzed:

1. Fifty persons dying with an acute infection, of these, 22, i. e., 44 per cent, showed venous plaques (not corrected for age).

2. Fifty-nine persons furnishing presumptive evidence of a disturbance of cholesterol metabolism in the direction of hypercholesteremia, viz., persons suffering from one or more of the following conditions: gallstones, obesity, diabetes, myxedema, nephrosis or biochemically proved hypercholesteremia. Of these, 28, i. e., 47.4 per cent, showed venous plaques. This higher incidence is, however, entirely due to the age distribution, and an analysis according to age groups shows that the

incidence of venous plaques is, if anything, smaller in this group than in the total series (table 2)

3 Thirty-one persons who had evidence of increased intravenous pressure Of these, 45 per cent showed venous plaques (not corrected for age)

TABLE 1—*Incidence of Venous Atheroma According to Age Groups*

Age Group	Persons in Group	Number with Plaques	Percentage with Plaques	Schilling's Percentage
0-20	30	8	26.7	
30-39	24	7	29.1	43.75
40-49	47	22	46.8	29
50-59	52	24	46.1	43
60-69	55	33	60	67
70-79	34	11	32.3	62
80-85	3	2	66	

TABLE 2—*Comparison of Rates of Incidence of Venous Atheroma in Age Groups of Whole Series and of Group with Cholesteremic Tendency*

Age Group	Percentage of Persons with Plaques	
	Whole Series	Group with Cholesteremic Tendency
41-45	38	14.5
46-50	59	50
51-55	50	40
56-60	40	50
61-65	64	64
66-70	54	50
71-75	30	43
76-80	40	40

TABLE 3—*Incidence According to Degrees of Atheroma of the Aorta*

Degree of Atheroma Aortae	Persons in Group	Number with Venous Atheroma	Percentage with Venous Atheroma
Minimal	19	6	31.5
Mild	53	24	45.2
Moderate	44	19	43.1
Marked	35	17	48
Severe	7	2	28
Total	158	68	43

Thus none of these three groups seems to be significantly linked with the occurrence of venous plaques

Finally 158 persons in whom the extent and the severity of aortic atheroma had already been determined were studied with regard to the incidence of venous atheroma For this purpose each was assigned to one of five groups as having minimal, mild, moderate, marked or severe aortic atheroma The result is summarized in table 3, which

shows that there is no correlation between the severity of aortic atheroma and the occurrence of the venous plaques

Schilling, too, was unable to establish any etiologic link between the occurrence of venous atheroma and the extent or the severity of the atheromatous lesions of the aorta. In his analysis there was a fairly well marked correlation between venous congestion and venous atheroma, 33 of 75 persons with venous plaques having suffered from venous congestion as against 13 of 75 persons without plaques. I was unable to confirm this relation.

ETIOLOGIC CONSIDERATIONS

Venous atheroma, speaking in general, is a great rarity. What, then, are the circumstances which produce it with considerable frequency in one particular location of the venous system?

A glance at the topography of the area of bifurcation will reveal the fact that here is the place of maximal mechanical stress in the whole venous system. The right common iliac artery crosses in front of the orifice of the left common iliac vein in such a manner as to compress this vessel once every systole between itself and the vertebral column¹⁸. There is no cushioning to lessen this effect.

The most impressive and often the only venous atheroma is found just at the center of this area of rhythmic compression. In addition, owing to the large diameter of the veins concerned and owing to the fact that the inferior vena cava possesses no valves, venous pressure at this point is also at its maximum. The arterial pressure exerted on the veins from the outside is also greater here than at the similarly built confluences of internal and external iliac veins (although even at these lesser bifurcations slight venous sclerotic changes are not infrequently found). It is in this region that the elsewhere fairly mobile inferior vena cava is firmly fixed to the aorta by connective tissue. Finally, here is a place where the returning venous stream changes its direction as it leaves the pelvis for the abdomen.

It is impossible to find a place in the whole venous system at which the vascular parietes are subjected to a greater or more constant mechanical stress.

18 The real nature of this compression can be gaged from the fact that in nearly every case the last half-inch of the left common iliac vein is widely dilated from the constant damming back of blood in it. Measurements taken in a great number of cases have shown that the proximal part of the left common iliac vein has a circumference on the average nearly twice that of the proximal part of the right common iliac vein. It forms a sort of venous sinus between the limbs of the arterial bifurcation. In the case of the left vena cava, however, the measurements were reversed, the circumference of the right common iliac vein being twice that of the left (3 vs 1.5 inches).

The cholesterol content of venous blood is as great as that of arterial blood,¹⁹ and if the instability of this substance were the essential cause of atheroma, one would expect atheroma to occur in many locations of the venous tree. A lesion as strictly localized as the one described in this paper must have a local cause and this I see in the mechanical stress to which the veins are subjected at this point. One case in which there was sinistroposition of the inferior vena cava and in which there was exhibited a mirror image distribution of the sclerotic lesion lends force to this opinion. It seems, therefore, that even if an instability of blood cholesterol were conceded as a necessary cause of atheroma that it can only exert its action in the presence of mechanical stress.

Schilling's conclusions about the causation of venous atheroma are similar. He said

Phlebosclerosis can exhibit the same degenerative changes (hyalinization, fatty changes, calcification) in the same degree as arteriosclerosis, but always only in locations which through special anatomical and functional circumstances are subjected to strong mechanical stresses

COMMENT

The finding of intimal thickenings containing intracellular and extracellular fat with splitting of elastica, hyalinization and calcification makes the identification of these lesions as atheroma secure. Whether the purely fibrous plaque occurring in the same location can also be regarded as atheroma is a matter of opinion. Fibrosis is a constant feature of arterial atheroma, and purely fibrous plaques do occur in arteries. If one insists on the demonstration of fat in every case before classifying it as atheroma the effect would be to reduce the incidence of venous atheroma from approximately 50 to approximately 25 per cent. The main argument would remain unaffected. Therefore one concludes that at least 25 per cent of all adults show venous atheroma. This atheroma is of a mild type and much more inclined to heal by fibrosis than its arterial counterpart.

The immunity of veins respecting atheroma argues that an essential atherogenetic factor is lacking in them. The fact that atheroma occurs at one particular spot of the venous tree with considerable frequency argues that this missing essential atherogenetic factor is present at that one spot in the venous system. The atheroma-bearing spot is found to differ from the rest of the venous system in being the point of maximal mechanical stress. This sums up to the following thesis

- (1) That the essential atherogenetic factor is the mechanical stress on the vascular wall,

19 Shillito, F. H., Bidwell, E. H., and Turner, K. B. *J. Biol. Chem.* **112** 557, 1935

- (2) That the immunity of veins as regards atheroma is not due to a different composition of venous blood nor to a different reactivity of the venous wall but to the absence of mechanical stress comparable to that acting on the arteries,
- (3) That where mechanical strain of comparable magnitude exists veins develop atheroma in much the same way as arteries do

The analysis of these cases of venous atheroma thus furnishes a further argument in favor of the widely held opinion that mechanical stress is the fundamental etiologic factor of atheroma. It must be clearly understood that in putting forward this etiologic proposition no pronouncements whatever are made about the pathogenesis of atheroma. How pressure works its effect, whether by upsetting the stability of blood cholesterol, whether by traumatizing the endothelium, whether by simply massaging an excess of plasma into the intima, etc., is a different question altogether. But in trying to answer these pathogenetic questions investigators should never lose sight of the basic etiologic factor, viz, pressure. It is in the belief that the pathogenetic researchers, especially of the "cholesterol" school of thought, are tending to obscure this fact that I present this contribution.

If venous atheroma is determined by local factors, it is not surprising that neither Schilling nor I was able to demonstrate any correlation between the severity of aortic atheroma and the occurrence of venous atheroma. However, in most cases of calcified venous atheroma (6 out of 8) there was also severe calcification of the aortic lesions. This suggests that while atheroma is largely determined by local conditions, a systemic factor is concerned in the production of grossly calcified atheroma. This idea gains support from the fact that in this small group of cases one found evidence of either metastatic calcification or some other upset of calcium metabolism. I intend to deal fully with this aspect of calcified atheroma in a separate paper.

SUMMARY

In almost half of all adults atheromatous plaques are found in a small area comprising the proximal end of the left common iliac vein and the medial distal 2 cm of the inferior vena cava. The fact that atheroma occurs at the point of maximal mechanical stress in the venous system should be taken into account in any reasoned assessment of the problem of atheroma.

UNUSUAL FORMS OF BLASTOMYCES DERMATITIDIS IN HUMAN TISSUES

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GILCHRIST¹ published the first description of blastomycosis in 1896. Since then, many cases of *Blastomyces dermatitidis* infection have been described.² These reports have illustrated the usual tissue findings in the different types of human blastomycotic infections. In the course of study of a case coming to autopsy at Duke University Hospital in which pulmonary blastomycosis complicated carcinoma of the gallbladder, many unusual forms of the organism were found. Some of these were smaller than any previously described in tissues and were so similar to *Histoplasma capsulatum* that they might easily have been confused with that organism.

The patient was a 76 year old white woman in whom chills, fever and a palpable abdominal mass developed in September 1948. A week later, after induction of ether anesthesia, cholecystotomy was done, with removal of many gallstones. Following the operation, the patient had persistent bile-stained abdominal drainage and periodic night sweats. She continued to fail, losing about 50 pounds (22.5 Kg.) of weight, until late January 1949, at which time the bile drainage ceased and jaundice promptly developed, with severe abdominal pain, anorexia, nausea and vomiting.

As a child she had typhoid, and in 1942 and 1944 tumors were removed surgically and with irradiation from her left ear and from below her eye, which were diagnosed as epithelioma. A deep, pitted wound in the side of her head, draining foul pus, persisted for the remainder of her life.

On entry she was heavily jaundiced and emaciated. Her left ear was absent, in its place was the crater of an ulcer approximately 1.5 cm. in diameter and 1 cm. deep, in the base of which was necrotic bone. Aside from this and the healed surgical scar her skin showed no lesions. Her lungs were clear. Her heart was normal. Her abdomen revealed a smooth and somewhat tender mass in the right upper quadrant which moved with respiration and seemed to be attached to the liver. Roentgenograms of the chest revealed moderate calcification in both hilar regions and linear radiation from the hilus into both upper lobes, suggesting scarring and perhaps old fibroid tuberculosis.

The patient seemed to be failing so rapidly, despite routine therapy, including transfusions, that surgical relief was attempted. Under local procaine anesthesia, the old operative incision was opened. About 300 cc. of thick, green pus was

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1 Gilchrist, T. C. Johns Hopkins Hosp. Rep. 1: 269, 1896.

2 Martin, D. S., and Smith, D. T. Am. Rev. Tuberc. 39: 275, 1939.

drained, several small gallstones were removed, and a biopsy was made of an irregular, nodular mass in the gallbladder, which revealed a well differentiated carcinoma. After the operation her condition remained poor, and despite supportive therapy, penicillin, transfusions and oxygen she gradually sank into a deep coma, became cyanotic and died quietly on the fifth day after the operation.

At autopsy the chest was emphysematous. In the right upper abdominal quadrant was a relatively fresh surgical wound from which semipurulent, bile-stained fluid drained. There were fibrinous pleural adhesions in the right side of the chest and more dense fibrous adhesions laterally and posteriorly in the left side of the chest. There was no fluid in either thoracic cavity. The abdomen contained about 600 cc of clear ascitic fluid. All of the organs were deeply jaundiced. A large mass was found in the region of the gallbladder, adherent to the skin and all neighboring viscera.

The right lung showed hemorrhagic, confluent lobular pneumonia in the lower lobe. The middle and upper lobes of the right lung and the entire left lung showed patchy irregular gray areas of variable size which felt much like pebbles embedded in the lung parenchyma. In addition to the large solid areas there were tiny granular foci resembling miliary tubercles scattered throughout. Little of the lung tissue looked normal, and crepitation was absent or minimal throughout. Hilar lymph nodes were scarred and calcified. The liver showed early cirrhosis and several small metastases, some of which had caused obstruction of the left main hepatic biliary duct. The gallbladder was incorporated into a dense fibrous and fatty mass adherent to the abdominal wall, the liver, the duodenum, the pancreas and the body wall. It showed a central empyema cavity with a necrotic inflammatory lining and an irregular annular lesion, diagnosed as carcinoma.

Postmortem cultures of material taken from various sites showed beta hemolytic streptococcus, *Escherichia coli* and *Klebsiella pneumoniae*. The cultures for fungus were unfortunately discarded when these organisms were identified.

Microscopic examination showed that the gallbladder was the seat of an adenocarcinoma which had metastasized to the liver, the head and tail of the pancreas, the left adrenal gland and to many abdominal lymph nodes. A single focus of microscopic size was found in the left lung.

The other abnormalities found in the lung proved to be granulomatous in nature. There were solid tubercle-like lesions with fibrosis and definite epithelioid cell and giant cell formation. There was, in addition, granulomatous pneumonia with extensive fibrosis, lymphocytic and polymorphonuclear exudate and, again, many giant cells. In many of the giant cells organisms were found varying from tiny granules, each surrounded by a vacuole, to very large solid forms, each showing a hyaline capsule. No endosporulation was noted, and budding forms were common. In no case were there multiple buds on a single organism. All transitions were found between the various forms noted, and in the fresh wet preparations the tiny forms also showed hyaline capsules.

The lesions removed from the left ear in 1942 and 1944 were reviewed, and they showed basal cell carcinoma. In addition, a granulomatous focus was found at one point, but no organisms could be identified in this tissue.

COMMENT

The relationship of carcinoma of the gallbladder and cholelithiasis has been well described, this case adds nothing to the literature on that account.

The lesions of the lungs show organisms of remarkably variable form. Large areas within a lesion show a tiny form of the organism and an inflammatory reaction made up of large mononuclear macrophages (fig 1), although the tiny form is found also in giant cells. The organisms resemble *H. capsulatum*, save that they are slightly larger, and the inflammatory reaction resembles that of histoplasmosis in many respects. The smallest forms of the organism appear to show the greatest shrinkage in preparation of histologic slides, and as a consequence more closely approximate the size of *H. capsulatum*. Forms measuring from 2.2 to 16.5 microns were found in the tissues. The organisms found in wet preparations made by scraping the cut surface of the lung and mounting in saline solution measured from 4.4 to 16.5 microns.

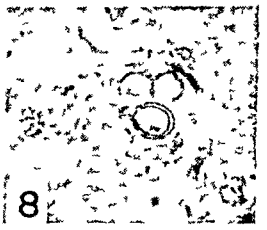
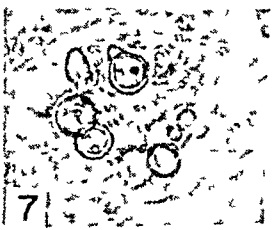
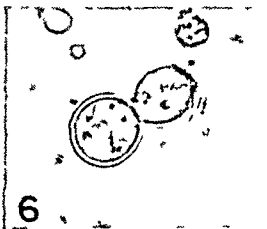
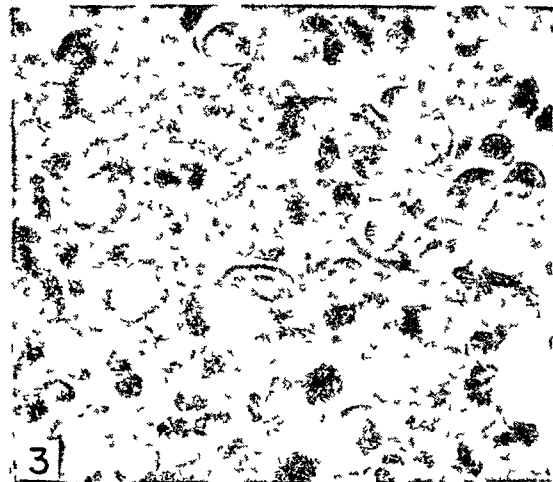
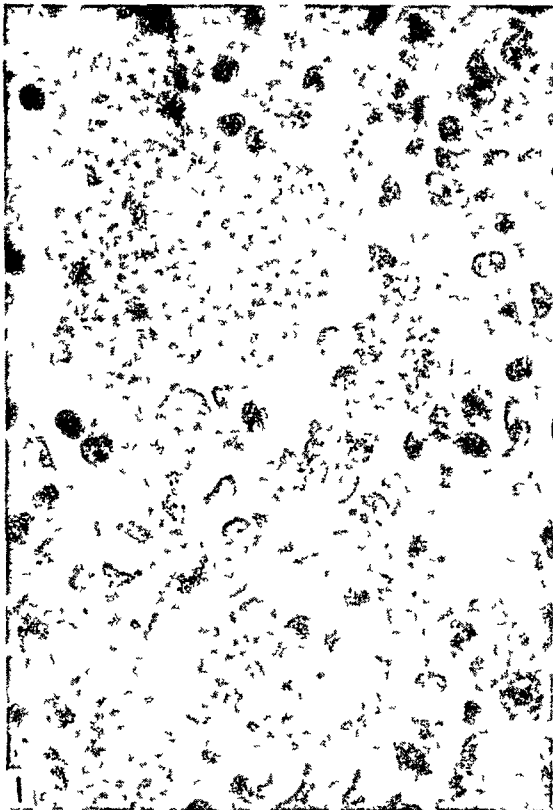
The cultural proof that these are *B. dermatitidis* is lacking. However, the characteristics of the organism are so clearcut that there is little room for question. The presence of budding forms, the absence of forms showing multiple buds, the relative thinness of the capsule in india ink preparations, the absence of endosporulation, the absence of mycelial growth in tissues and finally the size of the larger forms of the organism are all in accord with this diagnosis. In addition, the morphologic aspects of the smaller forms are identical with those of the others save for the size. The budding of the smallest forms is like that of *Blastomyces* and is not the constrictive type of the yeasts. The fact that all intermediate forms can be traced between the smallest and the largest organisms (fig 2) and that the smallest forms morphologically resemble *B. dermatitidis* is assurance that this is not a mixed infection. Furthermore, the larger organisms are identical with the common form of *B. dermatitidis* (fig 3), and the inflammatory reaction is a tubercle and giant cell-forming one like blastomycosis for the most part.

Small forms have been observed in cultures of this organism.³ Wade⁴ observed equally small organisms in a lesion of the skin, but the described characteristics differed markedly from the ones observed in this case.

The site of origin of the infection seems most likely to have been pulmonary. It is interesting to note with regard to systemic blastomycosis that the lungs are involved in over 90 per cent of the cases and are the chief organ involved in over 50 per cent of the cases. No relation could be found between the carcinoma and the blastomycosis save for the one tiny area in the lung where they coexisted.

3 Conant, N. F., Martin, D. S., Smith, D. T., Baker, R. D., and Callaway, J. L. *Manual of Clinical Mycology*, Philadelphia, W. B. Saunders Company, 1944.

4 Wade, H. W. *J. Infect. Dis.* **18**: 618, 1916.



Figures 1-9

(See legend on opposite page)

It is interesting to speculate on the possibility that there was a relationship between the debilitation produced by the carcinoma of the gallbladder, the jaundice and the patient's age, and the unusual picture of pulmonary blastomycosis found in this case. Further, it is considered possible that the ether anesthesia used in the first operation, five months before death, might have played a part in the development of the fulminating involvement seen in the lungs. However, the factor of virulence of the organism cannot be evaluated in this case.

SUMMARY

Forms of *Blastomyces dermatitidis* smaller than those previously recorded are described as observed in tissues.

The possibility that blastomycosis and histoplasmosis may be confused because of these small forms is emphasized.

EXPLANATION OF FIGURES 1-9

Fig 1—Small forms of *Blastomyces dermatitidis* in lung, hematoxylin and eosin, $\times 720$

Fig 2—Transition between the small and the usual forms of *B. dermatitidis* in lung, hematoxylin and eosin, $\times 720$

Fig 3—Forms of *B. dermatitidis* of the usually described type in lung, hematoxylin and eosin, $\times 720$

Fig 4—*B. dermatitidis* within giant cells in lung, hematoxylin and eosin, $\times 720$

Figs 5 and 6—Budding forms of organism, wet preparation from lung, $\times 720$

Figs 7 and 8—Small forms of organism showing budding, wet preparation from lung, $\times 720$

Fig 9—Usually described type of organism within a giant cell, wet preparation from lung, $\times 720$

URTICARIA PIGMENTOSA

A Report of a Case with Autopsy

JOHN M ELLIS, M D

MARTINEZ, CALIF

URTICARIA pigmentosa has been recognized as a clinical entity since Nettleship¹ described it in 1869 Unna² elaborated the histopathology of the lesions of the skin in 1887 by showing that there were dense accumulations of mast cells or tissue basophils in both the macular and the papular type This was subsequent to the work of Erlich,³ who had differentiated mast cells from the other connective tissue elements by the use of a toluidine blue stain which brings out the blue cytoplasmic granules Small numbers of these cells are scattered through normal tissue in man and other animals, and it is believed their relation to the blood basophilic cells is probably only morphologic Maximow, Downey and Ringoen⁴ concluded that the mast cells originate in connective tissue while the blood basophils are derived from the bone marrow The nucleus of the mast cell is single and round or oval, while that of the blood basophil is like that of the polymorphonuclear leukocyte

Recent interest in the role of the mast cell has been stimulated by Holmgren and Wilander⁵ and Jorpes, Holmgren and Wilander,⁶ who have concluded that the mast cell is the elaborator of heparin Bloom⁷ described mast cell tumors in dogs, and Oliver, Bloom and Mangieri⁸ assayed these tumors, finding their heparin content "50 times that of

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1 Nettleship Brit M J **2** 323, 1869

2 Unna, P G The Histopathology of the Skin, translated by N Walker, Edinburgh, New York, The Macmillan & Co, 1896, p 955

3 Erlich, P Arch f Anat u Physiol **3** 166, 1879, **4** 571, 1879

4 Maximow, A Arch f micr Anat **67** 680, 1906, **83** 247, 1913 Downey, H Folia haemat **16** 49, 1931 Ringoen, A R Am J Anat **31** 319, 1923

5 Holmgren, H J, and Wilander, O Ztschr f mikr-anat Forsch **42** 242, 1937

6 Jorpes, J E, Holmgren, H J, and Wilander, O Ztschr f mikr-anat Forsch **42** 279, 1937

7 Bloom, F Arch Path **33** 661, 1942

8 Oliver, J, Bloom, F, and Mangieri, C J Exper Med **86** 107, 1947

dog live! ” Lately there have been reports of large numbers of mast cells in the victims of the atomic bomb dying approximately one month after exposure⁹ There was a widespread hemorrhagic state in these victims¹⁰ and also in the test animals at Bikini,¹¹ whose blood when titrated with antiheparin substances seemed to indicate that an anticoagulant was present¹² This was partial confirmation of the work of Allen and Jacobson,¹³ who exposed dogs to total body ionizing irradiation They expressed the belief that their experimental evidence shows that the substance causing the hemorrhages is heparin

A classic study of urticaria pigmentosa was presented by Little,¹⁴ who reviewed all the cases (154) reported up to 1905 In 1923 Finnerud¹⁵ reviewed 152 additional cases, and since that time from one to five reports have been published each year, usually describing some unusual feature of the disease However, there has never been report of an autopsy as far as can be determined by a search of the available English, German and French literature The absence of such a report might be considered extraordinary in view of the relative frequency of urticaria pigmentosa except for the fact that the disease has been thought nonfatal Ormsby¹⁶ stated that “the active reproduction of the lesions commonly subsides spontaneously after a certain number of years ” This is substantially the opinion of all authors of standard textbooks of dermatology

The purpose of this paper is to present the case of a Negro girl born with macular and papular lesions later demonstrated to be histologically identical with those of urticaria pigmentosa The child died at the age of 12 months and 19 days The clinical history, the gross autopsy findings and a comparative histologic study are presented in that order

REPORT OF CASE

C M C was born in this hospital, July 5, 1946, after nine months of pregnancy Seven months prior to the birth the mother was found to have a purulent discharge of the cervix Smear and culture showed gonococci Syphilis was ruled out clinically, and the serologic tests were negative prior to and after the administration of 200,000 units of penicillin, which resolved the gonorrhea

The infant's birth weight was 2,800 Gm Her respiration was spontaneous Her lusty cry and physical condition were declared good Beneath the vernix caseosa were elevated nummular grayish nodular lesions on forehead, thighs and

9 Liebow, A A, and Warren, S Am J Path **23** 888, 1947

10 LeRoy, G T J A M A **134** 1143, 1947

11 Tullis, J L Am J Path **23** 891, 1947

12 Cronkhite, E P Am J Path **23** 891, 1947

13 Allen, J G, and Jacobson, L O Science **105** 388, 1947

14 Little, G Brit J Dermat **17** 355, 1905, **18** 16, 1906

15 Finnerud, C W Arch Dermat & Syph **8** 344, 1923

16 Ormsby, O S A Practical Treatise on Diseases of the Skin, Philadelphia Lea & Febiger, 1937, p 153

back, measuring 0.5 to 1 cm (fig 1). Some had a semitranslucent appearance, but all were firm, solid lesions. In addition, there was a diffuse reddish purple elevated rash on the face and the thorax.

A specimen of skin was taken for biopsy on August 7. The patient was sent home on August 9 with a note, "she is clinically well and gaining weight."

The routine hematoxylin and eosin stain of the skin taken for biopsy showed numerous inflammatory cells of the monocytic series in the corium. No specific diagnosis was made. The biopsy slides were submitted by Dr. H. V. Allington at

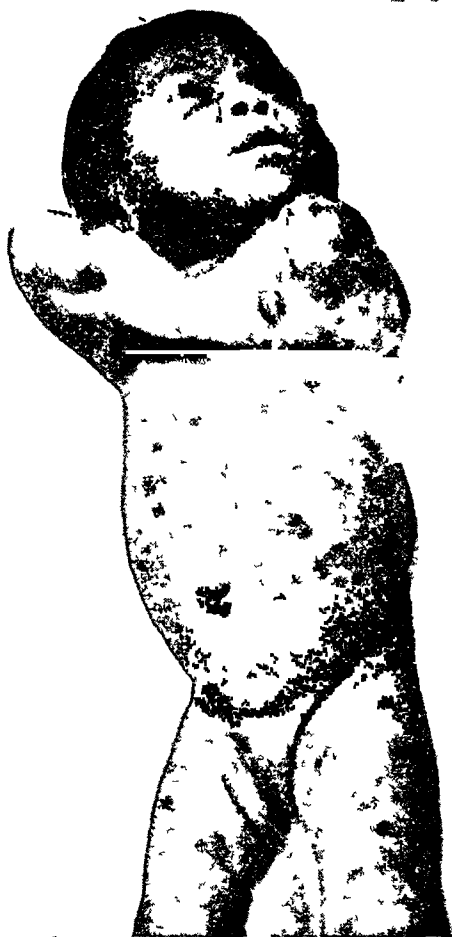


Fig 1—Lesions of the skin at birth

a dermatologic meeting, where it was suggested that the possibility of urticaria pigmentosa be considered.

The biopsy material was then stained with Giemsa stain and azure II, which revealed that a large portion of the cells infiltrating the corium were mast cells. The patient was readmitted to the hospital, April 10, 1947, with a chief complaint of swelling of the abdomen for the preceding two months and the skin rash which had been present since birth. The mother had given the child $\frac{1}{2}$ teaspoon of castoria® daily for five months but did not explain why. The baby had had three soft, yellow-green bowel movements each day and had scratched herself

for the preceding three months, but the rash was described as lessened in amount since the previous admission

The family history revealed that there was one other child, aged 3 That child, the father and the mother were all living and well There was no known tuberculous exposure

Examination showed many pigmented spots over the body The anterior fontanel was open and large The ear drums and canals were clear No rigidity or masses were noted in the neck The pupils were round and regular and reacted to light and distance The chest was normal to percussion, no adventitious sound

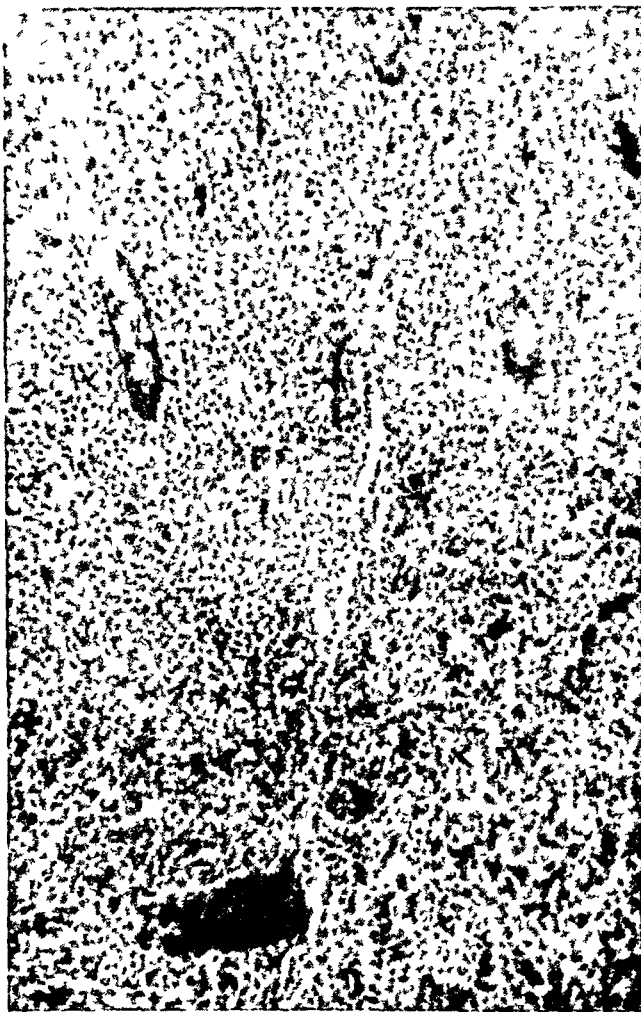


Fig 2—The mast cell infiltration of the corium

was heard The heart rhythm was regular, the rate, 110, no murmurs were heard The abdomen was swollen, tense and tympanitic The liver extended 4 cm below the right costal margin The spleen was questionably palpable Hyperactive peristalsis was noted The extremities were wasted, with no paralysis, deformities or edema, the reflexes were normal

The stools were voluminous and foul smelling The baby was restricted to a low fat, low residue banana diet but ate poorly, and in a few days diarrhea developed She was treated with penicillin, 10,000 units intramuscularly every three hours, until the diarrhea cleared up, as it did after a short period Her

temperature varied between 98 and 100.2 F without a characteristic curve. On June 21 liver was taken for biopsy and the hematoxylin, eosin stain showed early portal cirrhosis. There was no evidence of lipid infiltration. Previously, on May 22, a marrow smear showed active hemopoiesis with no evidence of infiltrating abnormal cells. On July 24 the patient died.

Roentgen examination (barium sulfate enema) showed marked looping of the descending colon. The lumen was dilated, but not extremely. Roentgenograms of the skull were negative for Hand-Christian-Schuller disease.

The Kline test was negative. The hemoglobin content on admission was 8.6 Gm, the count of white blood cells was 16,700, with 45 segmented cells, 8 band cells, 5 metamyelocytes, 40 lymphocytes and 2 monocytes per hundred. The urine was alkaline, with a faint trace of albumin, there was no sugar. The icteric index was 2. A red cell fragility test showed hemolysis starting at 0.46 and complete at 0.28 per cent sodium chloride. Blood cholesterol was 88 mg per hundred cubic centimeters. Titration of the gastric contents showed free hydrochloric acid 0 and total hydrochloric acid 6. Duodenal drainage showed amylase totaling 256 Somogyi units. A dextrose tolerance test showed fasting blood sugar, 80 mg, one hour, 116 mg, two hours, 136 mg. Multiple stool specimens revealed no fat droplets. Cultures of the stools showed no pathogenic organisms and were repeatedly negative for ova and parasites. Five days before death the hemoglobin was 11.6 Gm, and the blood counts were 4,030,000 red cells and 12,100 white cells, with 21 segmented cells, 4 band cells, 3 metamyelocytes and 72 lymphocytes per hundred. Anisocytosis and achromia were noted.

Autopsy—The body was that of a well developed, emaciated Negro girl 60 cm in height and 5,200 Gm in weight. A moderate amount of mucopurulent fluid was noted about the nares. No peripheral lymphadenopathy was found. The right lung weighed 50 Gm, the left, 40 Gm. The pleural cavities were dry and the lungs free. The lower lobes and the dependent parts of the upper lobes were purplish and discolored, and had depressed areas in a patchy distribution on the pleural surface and on cut section. A small amount of frothy fluid could be expressed from the surface. The lungs were subcrepitant. The heart weighed 20 Gm. The pericardial sac contained about 5 cc of a clear serous fluid. The pericardium was smooth and glistening and the myocardium moderately firm. The valves and the coronary arteries were normal. The thymus was small, atrophic, and there was a large number of greatly enlarged mesenteric and retroperitoneal lymph nodes of fairly uniform size, up to 1 cm in diameter. These were soft and discrete and cut with ease, showing a uniformly pale, smooth surface. The liver weighed 320 Gm. Glisson's capsule was glistening, and the surface had an ill defined, mottled appearance with slightly rounded edges. On cut section, many stellate, grayish white areas were noted in portal spaces. The gallbladder was normal. The spleen weighed 55 Gm, was firm and adhered to the diaphragm by delicate fibrous adhesions. The capsule was slightly thickened and gray, with the cut section showing gray-white stellate areas in the pale parenchyma. The pancreas and the adrenal glands were grossly normal. The gastrointestinal tract showed no lesions. The right kidney weighed 40 Gm and the left, 40 Gm. Both kidneys were deformed, the upper poles being depressed by the liver, the encroachment of which formed flat surfaces directed cranially and medially. The capsules stripped with ease. Their surfaces and cut sections were normal, as were those of the ureters and the bladder. The brain weighed 700 Gm and showed no abnormalities.

Mast Cell Counts—Tissues from various organs were fixed in a 4 per cent formaldehyde solution and stained with hematoxylin and eosin. Comparable sections were made from the tissues of normal infants of the same age because of the gradual decrease of normal mast cell counts with increasing age. All tissues were then stained with Giemsa stain after the method of Parsons and Black,¹⁷ and comparative counts were made of the numbers of mast cells in the tissues of the patient as well as in those of the controls (see table). The counts tabulated represent the average number of mast cells per oil immersion field seen in the tissues of the patient as compared with the control counts. Mast cell counts were made in 10 cases of portal cirrhosis and 10 cases of Hodgkin's disease, and in no case was an increase found.

•*Description of Mast Cells Observed*—Two kinds of mast cells were seen. One had deeply stained metachromatic granules closely packed within the cytoplasm. The second had fewer, more widely spaced granules, which appeared to take up less dye, as though they had not reached or had gone beyond their maturity.

In some areas of the liver, where the mast cells were most numerous, it was possible to identify the basophilic cytoplasmic granules in the stroma beyond the cell walls. Cells having the same morphologic aspect as mast cells were seen in the sinusoids of the lymph nodes, spleen and liver and, in small numbers, in various other vascular channels in other tissues. The mast cells varied from 10 to 25 microns. Some were spherical, while others were stellate or lancet shaped. The nuclei were usually ovoid, but those of the stellate or lancet form were highly irregular. No morphologic difference could be noted between those of the patient and those of the controls.

Weigert's method of staining the tissue did not reveal the inclusion bodies described by Bloom⁷ in his canine cases of mast cell tumor.

Microscopic Description of the Tissues—The liver showed extreme widening of the portal areas by connective tissue, which varied from adult collagenous to fibroblastic type. There was striking hyperplasia of the small bile ducts. In the areas of fibroblastic proliferation there were lymphocytes, plasmacytes, an occasional macrophage and eosinophilic granulocyte as well as large numbers of tissue mast cells. There were from 25 to 30 of these mast cells in each oil immersion field of the areas of fibrosis and from none to 2 mast cells in the undamaged portions of the parenchyma. Usually one border of the mast cell was in apposition to a sinusoid, but occasionally the cell was completely surrounded by parenchymal cells. An occasional mast cell was seen within the lumen of a sinusoid.

The periportal fibrosis seen in the biopsy specimen was not as marked as that present at the time of death. There was less hyperplasia of bile ducts and fewer, more immature mast cells in the fibrotic areas than at autopsy.

In the lung, marked intra-alveolar hemorrhage was found. Colonies of bacteria were observed in the bronchi and in the alveoli. There was patchy bronchopneumonia.

Two to 5 mast cells were seen in each oil immersion field of the pleura and the septal connective tissue. One to 3 mast cells could be seen in each oil immersion field of the parenchyma, chiefly in the alveolar wall and occasionally in an alveolus.

The splenic capsule and the trabeculae were very thick. The white pulp was diminished, and the germinal centers of the splenic corpuscles were not visible. The red pulp was moderately hyperemic, with large numbers of eosinophilic cells scattered throughout. There were 10 to 25 mast cells in each oil immersion field.

of the red pulp, but none was seen in the white pulp or in the capsule or the trabeculae. There were many areas in which fibrosis had taken place. Fibroblastic tissue was seen in the vicinity of older fibrosis.

The bone marrow immediately beneath the endosteum contained large numbers of mast cells and eosinophilic cells. There were 50 to 60 mast cells per oil immersion field in the marrow adjacent to the endosteum, with approximately 1 mast cell to each of all other types of marrow cells. The ratio of mast cells to other types of cells fell as one examined deeper into the marrow and away from the endosteum.

In the biopsy of marrow no mast cell or other abnormalities were seen.

The kidney revealed acute passive congestion, with many small hemorrhages of the medulla between the collecting tubules. From 2 to 5 mast cells were seen in each oil immersion field in the interstitial connective tissue surrounding the convoluted tubules and the loops of Henle in the cortex. No mast cells were seen in the medulla. From none to 3 mast cells per oil immersion field were

Mast Cell Counts (Average Numbers per Oil Immersion Field)

Tissue	Tissues of Normal Controls		Tissues of Patient	
	Parenchyma	Capsule or Surrounding Connective Tissue	Parenchyma	Capsule or Surrounding Connective Tissue
Liver	0-1		25-30	
Lung	1-2		2-5	
Spleen	0		10-25	
Thymus			50-60	
Bone marrow	0		50-60	
Kidney	0-1		2-5	
Pancreas	1-2		30-40	
Urinary bladder	0-3		0-2	
Ovary	0	0-1	0	0-4
Adrenal gland	0	0	0	3-5
Pituitary gland (anterior lobe only)	0	0-1	0	3-9
Cerebrum	0		0	
Cerebellum	0		0	
Lymph node 1	0	0-1	20-30	10-15
Lymph node 2	0		20-30	10-15
Lymph node 3	0		10-15	10-15

seen in the intercapillary spaces of the glomerular tuft, but these were not numerous.

In the pancreas there was extreme interstitial fibrosis with a relatively small amount of acinous tissue, which appeared to be undergoing atrophic changes. The connective tissue in many places was heavily infiltrated with lymphocytes, plasmacytes, histiocytes and mast cells. In many of the areas of interstitial fibrosis there were as many as 40 mast cells per oil immersion field, and in a few there were as many as 5 to 10 eosinophilic cells.

Acute passive congestion was noted in the urinary bladder. Only a very occasional mast cell was seen in the submucosa and the interstitial fibrous connective tissue of the muscle layers.

The ovary presented a normal histologic picture throughout. There were 1 to 4 mast cells per oil immersion field in the connective tissue of the hilus. No mast cell was seen in the parenchyma.

The adrenal gland had normal parenchyma except for slight hyperemia. There were several small hemorrhagic areas surrounding the adrenal gland, in the fat. Also, there were several islands of persisting fetal fat around the gland. No mast cells were seen in either the medulla or the cortex. There was from none

to 1 mast cell per oil immersion field in the capsule and from 3 to 5 mast cells in the fetal fat

In the pituitary gland (anterior lobe only) there were several small hemorrhages in the capsule and in the parenchyma. The parenchyma was moderately hyperemic, but otherwise the structure was well preserved. There were 3 to 9 mast cells per oil immersion field in the true capsule and in the surrounding connective tissue. No mast cells were seen in the parenchyma.

Mast cells were not seen in the meninges or within the brain substance.

The capsule of a mesenteric lymph node (lymph node 1 in table) was thickened and the node enlarged. The germinal centers were present in normal numbers but were small, and the lymphoid cells were few. Very few mast cells were found in the germinal centers. The lymphoid cells in the reticulum were largely replaced by eosinophilic and mast cells, with 20 to 30 of the latter in each oil immersion field. The small blood vessels were widely dilated. Mast cells were scattered throughout the capsule and the trabeculae in about one-half the number found in the reticular areas. An occasional mast cell typical of those seen in the tissue was found in the lumen of a peripheral sinus.

Lymph node 2 was essentially the same as that described in the foregoing paragraph, in the general histologic aspect and in mast cell content.

Lymph node 3 also showed the same general histologic picture with about one-half the number of mast cells in the parenchyma and approximately the same number of mast cells in the capsule and the trabeculae.

The table shows comparative counts of the mast cells observed in various organs and tabulated as average numbers per oil immersion field. The controls were infants of the same age dying of traumatic lesions. There is an absolute increase in the number of mast cells in each organ in this case of urticaria pigmentosa as compared with the controls. This demonstrates urticaria pigmentosa as a systemic as well as a cutaneous disease.

COMMENT

The ultimate cause of death appears to have been pulmonary edema with aspiration of stomach contents, followed by early bronchitis. These features seem to be the sequelae of extreme cachexia.

As regards the underlying pathologic process leading to death, several possibilities were considered and ruled out clinically by laboratory examination or roentgenography. Hodgkin's disease was considered carefully, and there were histologic evidences of it, i. e., increased numbers of eosinophils and some fibrosis. No Reed-Sternberg cells could be demonstrated. However, Jadassohn¹⁸ has taken simultaneous specimens for biopsy from patients with urticaria pigmentosa—one from a nonirritated lesion and one from a lesion irritated by scratching. The latter showed mast cells intermixed with large numbers of eosinophils. In the former eosinophils were absent. Mast cells were not present in significant numbers in any of the 10 cases of proved Hodgkin's disease in which tissues were examined in the course of this study.

Histologically, the liver shows portal cirrhosis, but cirrhosis is a rare disease in infancy, and too there were huge numbers of mast cells

18 Jadassohn, W. Arch f Dermat u Syph 167 704, 1933

in the liver of this infant which were not present in 10 cases of portal cirrhosis surveyed in this study. Sutton¹⁹ searched the American literature in 1930 and found 12 cases of cirrhosis in childhood without a history of alcoholism and 10 cases with such a history. It is not possible to say more of the cirrhosis except to call attention to the parallelism in this patient and in Bloom's⁷ dogs with mast cell tumors. Their skin lesions were similar. There were mast cells in the lung, bone marrow and periadrenal tissue and, most strikingly, the dog's livers exhibited "parenchymatous degeneration with periportal fibrosis and solitary and nodular collections of mast cells." The liver at biopsy as compared with the liver at autopsy contained fewer mast cells and less fibrosis and the nuclei of the mast cells were generally more vesicular, with the cytoplasmic granules more scattered and smaller. Paff, Bloom and Reilly,²⁰ who had followed mast cells developing in tissue cultures, stated that these less granular forms are immature.

Study of the autopsy material showed many points of similarity to cancer. There were multiple cutaneous tumors showing dense collections of mast cells with a certain pleomorphism as described. These cells were found in huge numbers in mesenteric lymph nodes, spleen, liver and bone marrow. There was a one to three fold increase over the number of mast cells seen in connective tissues of the controls. Mast cells were found quite frequently in the peripheral sinuses of the spleen. If the mast cells do, indeed, have extramylloid origin as contrasted with the blood basophils, then the mast cells focally concentrated in the marrow of this patient must have been carried there by a metastatic process.

Touraine, Solente and Renault²¹ have classified urticaria pigmentosa as a pseudoleukemia, citing the splenomegaly and lymphoid hyperplasia as evidence. They did not present evidence from biopsy of the spleen or the lymph nodes but stated that these are often clinically enlarged. It may be noted here in passing that Fabris²² and Schreus²³ have produced mast cell tumors in mice by applying tar to the skin. This is consistent with the tar induction of certain other neoplastic diseases.

The significance of this study appears to be that it demonstrates urticaria pigmentosa as a systemic and not a purely dermal disease as it has been considered by many. The disease might be compared with lupus erythematosus in this regard. If this case can be considered one

19 Sutton, T. L. *Am J Dis Child* **39** 141, 1930

20 Paff, G. H., Bloom, F., and Reilly, C. *J Exper Med* **86** 117, 1947

21 Touraine, Solente and Renault, P. *Bull Soc franç de dermat et syph* **40** 1691, 1933

22 Fabris, A. *Pathologica* **19** 157, 1927

23 Schreus, H. T. *Dermat Ztschr* **40** 9, 1924

of a true cancer in the classic sense, then it appears to be the first reported human case and may be compared with the canine cases of Bloom⁷

Another interpretation must be considered, namely, an alteration of physiology. If it could be assumed that the mast cell is the originator of heparin, it would be reasonable to assume that there was some mechanism calling for an increased production of heparin. This was present in utero inasmuch as the tumors were present at birth. The gonococcic infection that was found in the cervix during the second month of pregnancy appears to have been the only untoward incident in the puerperium. Coincidentally, mast cells may be found in greatly increased numbers surrounding areas of chronic inflammation such as infected pilonidal cysts and fistula in ano.

Résumé of Comment—What appears to be the first report of an autopsy made in a case of urticaria pigmentosa has been presented. Some light has been shed on the systemic occurrence of the disease, which heretofore has been lacking. The cause is not established by this study, nor is the relation of the disease described to classic urticaria pigmentosa absolutely clear. In cases of the latter the patient usually survives to die of some other disease after the urticaria has regressed. This patient's disease may be an example of cancerous mastocytoma similar to the mast cell tumors described by Bloom in dogs, and may confirm the hypothesis of Touraine, Solente and Renault that urticaria pigmentosa is a pseudoleukemia, or it could represent the histologic response to an alteration of physiology, pointing to the gonococcic cervicitis that was discovered during the second month of pregnancy.

SUMMARY

A study is presented of an infant dying in the active phase of urticaria pigmentosa. This appears to be the first instance in which a study of the internal lesions of this disease has been made.

Increased numbers of mast cells were found in many portions of the body, with special attention being drawn to the lymph nodes and bone marrow, where the collections of cells simulate metastatic lesions. The liver showed a process of cirrhotic fibrosis bearing a similarity to the processes described by Bloom in canine cases of mast cell tumor.

The lesions of the skin were congenital, and the mother had gonococcic cervicitis in the first trimester of carrying this infant. Numerous mast cells are to be seen in the vicinity of many chronic infections. These facts may be related, and it can be postulated that this infant's congenital lesions might be the result of a localized demand for heparin due to the adjacent cervicitis.

VIRUS-LIKE GLOBULES IN CANCER EXTRACTS

Electron-Microscopic Studies of Thirty Human Tumors

C ALEXANDER HELLWIG, M D

WICHITA, KAN

IN A REVIEW of the literature concerning the properties of cancer cells Cowdry¹ in 1940 concluded that there was no reliable basis on which to reach a decision as to whether a single cell observed in a section of suspected tissue is or is not "malignant," and he stated that there is no satisfactory evidence that "malignant cells" of man possess anything—virus or otherwise—which is wholly absent in normal cells. Both statements seem to be refuted by recent studies made with the electron microscope.

In 1947 Claude, Porter and Pickels² observed in the cytoplasm of chicken tumor cells small spherical bodies ranging in diameter from 67 to 80 millimicrons. These were interpreted as the transmitting agent of chicken sarcoma. At the same time, Porter and Thompson³ published electron micrographs of rat sarcoma cells which showed similar dense granules with a diameter of from 50 to 250 millimicrons. While the authors hesitated in suspecting that specific virus particles were seen in these globules, they regarded them as morphologic characteristics of "malignant cells."

In 1948 Porter and Thompson⁴ observed spherical bodies which had an average diameter of 130 millimicrons in cultured mouse carcinoma cells. The uniform morphologic aspect and association of these bodies in closely packed clumps suggested to them that the bodies were of extraneous origin and probably represented the virus-like milk factor. In size the particles observed by Porter and co-workers differed greatly from the globules described by English investigators Passey, Dmochowski, Astbury and Reed⁵ treated tissue extracts with

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From the Electron Microscope Laboratory of the Sedgwick County Tumor Clinic and St Francis Hospital

1 Cowdry, E V Arch Path **30** 1245, 1940

2 Claude, A , Porter, K P , and Pickels, E G Cancer Research **7** 421, 1947

3 Porter, K R , and Thompson, H P Cancer Research **7** 431, 1947

4 Porter, K R , and Thompson, H P J Exper Med **88** 15, 1948

5 Passey, R D , Dmochowski, L , Astbury, W T , and Reed, R Nature, London **160** 565, 1947

benzin and trypsin and filtered them through a Berkefeld filter. They found in such extracts of tumors of mice of high breast cancer strains spherical particles with a diameter of only 20 millimicrons.

Using a high speed microtome which allows the cutting of tissue sections 0.1 micron thick, Gessler and Grey⁶ demonstrated in human cancer tissue spherical bodies ranging from 80 to 150 millimicrons. Since there was a close similarity between these globules and the virus of fowl sarcoma, they supposed that they were probably dealing with virus-like causative agents of cancer cells.

Summary of Electron-Microscopic Observations

Patient	Age	Sex	Histologic Diagnosis	Method of Preparation	Size of Globules, Millimicrons
1	30	F	Melanoma	Maceration in water	20-95
2	54	F	Melanoma	Pepsin digestion	30-60
3	66	M	Carcinoma of bladder	Pepsin and trypsin digestion	Absent
4	22	F	Melanoma	Pepsin digestion	Absent
5	40	F	Medullary carcinoma of breast	Trypsin digestion	75-95
6	55	F	Metastatic carcinoma of ovary	Trypsin digestion	80-108
7	49	F	Duct cell carcinoma of breast	Trypsin digestion	30-105
8	39	F	Squamous cell carcinoma of vulva	Trypsin digestion	53-160
9	50	F	Duct cell carcinoma of breast	Benzin-water extract	60-108
10	68	F	Metastatic adenocarcinoma in lymph node	Saline extract	53-120
11	64	F	Duct cell carcinoma of breast	Benzin-saline extract	55-160
12	59	M	Hodgkin's disease	Benzin-saline extract	27-60
13	56	F	Metastatic melanoma in lung	Benzin-saline extract	80-120
14	48	F	Duct cell carcinoma of breast	Benzin-saline extract	55-175
15	49	M	Hodgkin's disease	Maceration in water	33-67
16	62	F	Squamous cell carcinoma of cervix	Water extract	40-80
17	66	F	Squamous cell carcinoma of cervix	Pepsin digestion	27-54
18	65	F	Anaplastic carcinoma of bladder	Pepsin digestion	33-67
19	30	F	Melanoma	Pepsin digestion	53-93
20	67	M	Papillary carcinoma of rectum	Acetic acid	53-100
21	68	M	Papillary carcinoma of bladder	Water extract	27-40
22	36	M	Melanoma	Water extract	56-95
23	36	F	Adenosarcoma of uterus	Water extract	28-88
24	30	F	Chronic cystic mastitis	Benzin-saline extract	40-80
25	38	F	Fibroadenoma of breast	Benzin-saline extract	60-95
26	18	F	Fibroadenoma of breast	Benzin-saline extract	33-73
27	23	F	Fibroadenoma of breast	Benzin-saline extract	27-60
28	42	F	Meningioma	Benzin-saline extract	40-55
29	46	F	Fibroadenoma of breast	50% acetic acid	5-80
30	64	F	Lymphadenoid goiter	Saline extract	25-60

The importance of these electron-microscopic observations cannot be overemphasized. Should it be possible to see these particles in cancers of human origin in routine examination, the hundred year old problem of the "single cell" diagnosis of cancer (Hellwig,⁷ 1932) would be solved.

MATERIALS AND METHODS

Twenty-three cancerous and 7 benign tumors of human origin have been studied during the last two years in the laboratory of the Sedgwick County Tumor Clinic and St. Francis Hospital with the electron microscope. Different methods of preparing tissue extract were tried, for instance, maceration in water, crushing

⁶ Gessler, A. E., and Grey, C. E. *Exper. Med. & Surg.* **4**: 307, 1947. Gessler, A. E., Grey, C. E., and McCarty, K. *ibid.* **6**: 329, 1948.

⁷ Hellwig, C. A. *Arch. Path.* **13**: 607, 1932.

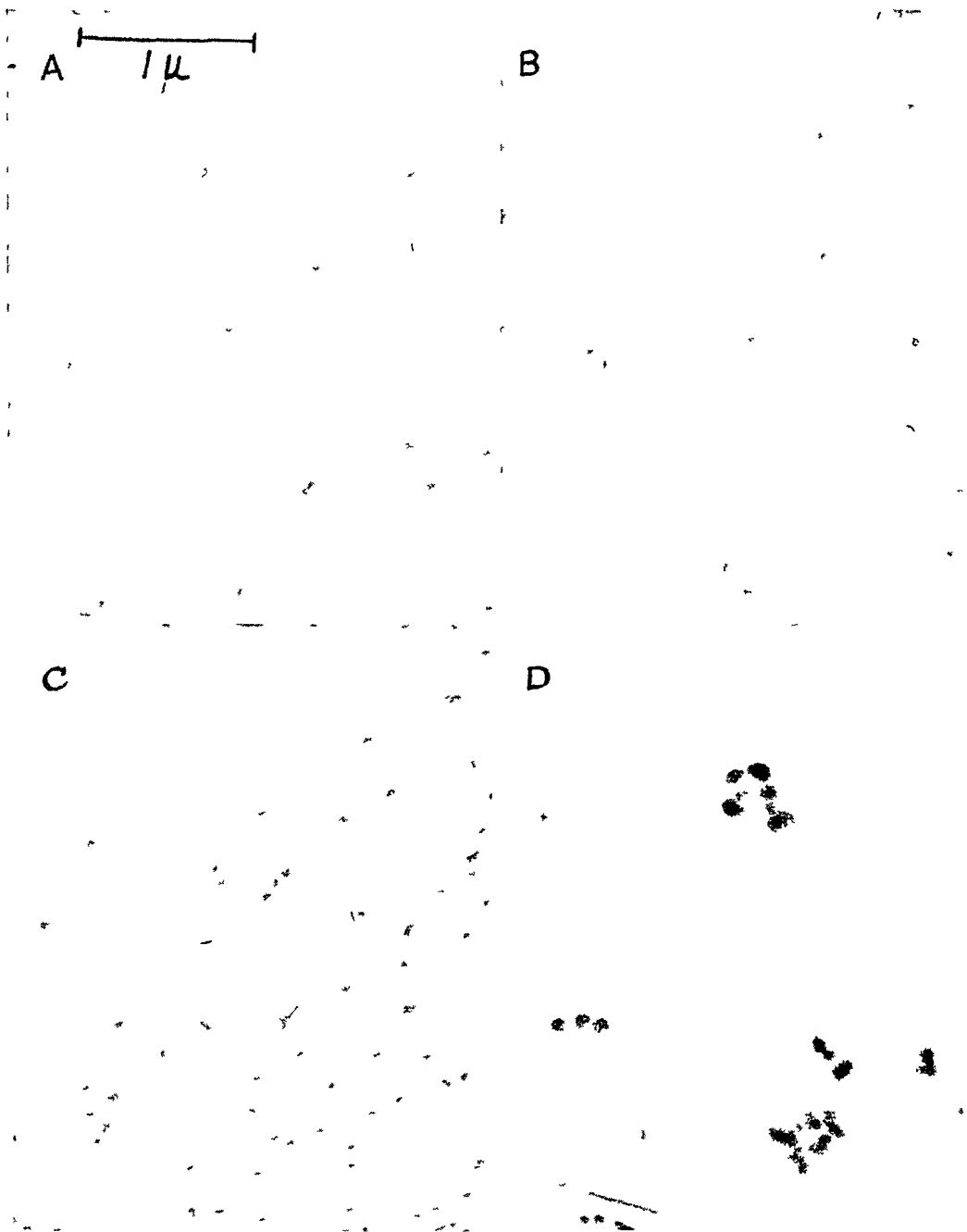


Fig 1—*A*, lymphadenoid goiter (case 30), small spherical globules forming chains and clusters *B*, meningioma (case 28), small spherical particles measuring between 40 and 50 millimicrons *C*, Hodgkin's disease (case 15), small globules and some aggregates of larger micelles *D*, carcinoma of breast (case 11), large globules in pairs and short chains

These electron micrographs were taken at a magnification of 5,000 diameters and photographically enlarged 4 times, so that final magnification is 20,000

of tissue in acetic acid, digestion with pepsin and trypsin and extraction with saline solution. Finally the following method was accepted as the routine procedure. Fresh tumor tissue was placed in the mincing apparatus devised by Carpenter⁸, 0.1 cc of the pressed juice thus obtained was mixed in a graduated centrifuge tube with 10 cc of petroleum benzine USP. The suspension was left standing for thirty minutes in the refrigerator, then the benzine was poured off and the sediment was mixed with 10 cc of isotonic sodium chloride solution. The mixture was left in the refrigerator overnight (eighteen hours), then the tube was centrifuged at 3,000 revolutions per minute for thirty minutes. Four to six samples were taken from the supernatant fluid of each tube. A drop was placed with a micropipet on a collodium film supported on a 200 mesh wire screen. The sample was allowed to dry in the air and was kept in a desiccator over calcium chloride until ready for examination. The original micrographs were taken with

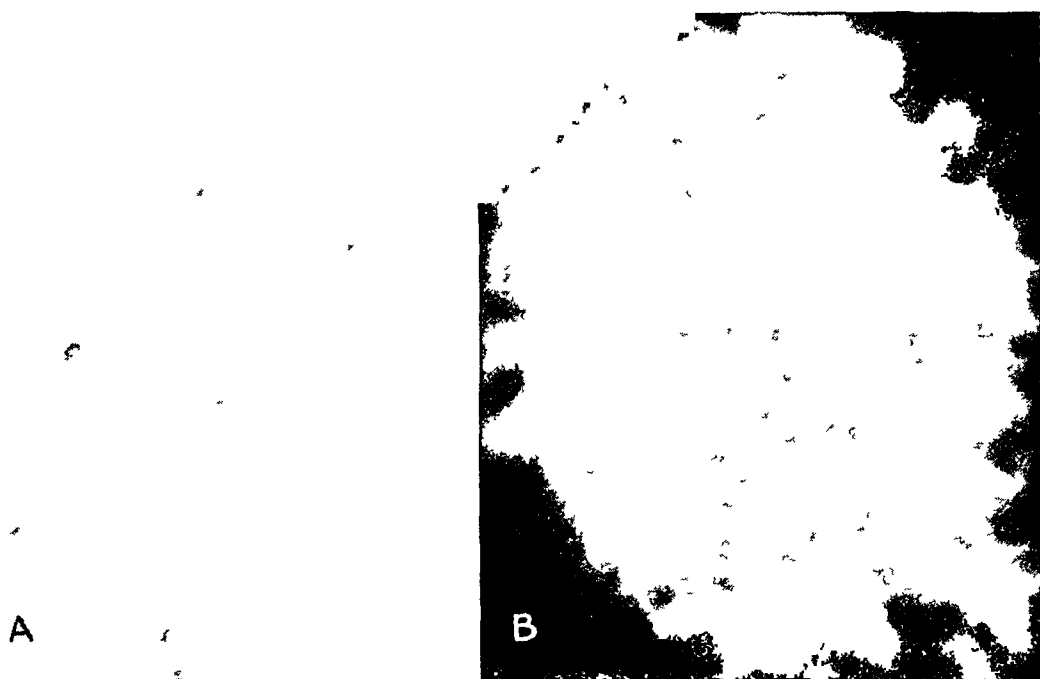


Fig 2—*A*, melanoma (case 19), single globules of large diameter together with smaller globules, pepsin digestion extract. *B*, ovarian carcinoma (case 6), large globules, single and in short chains.

These electron micrographs were taken at a magnification of 5,000 diameters and photographically enlarged 4 times, so that the final magnification is 20,000

an RCA electron microscope on Eastman contrast lantern slides at a magnification of 5,000, and the negatives were studied in a Spencer microfilm reader, which magnifies fifteen times. Thus a total magnification of 75,000 diameters was obtained.

OBSERVATIONS

The table summarizes the electron-microscopic observations together with the histologic diagnosis, the age and the sex of each patient.

Globular bodies were noticed in most extracts of tumors, whether these were cancerous or benign. Their diameter ranged from 20 to 160 millimicrons. They

8 Carpenter, E. Science **106** 621, 1947

were spherical, and in most of the extracts they were well separated from one another. In several specimens, clusters, short chains and larger aggregates were present. There was a definite difference in size between cancerous and benign tumors. As a rule the size of the globules was larger in cancer tissues than in benign tumors. While in extracts of benign tumors the particles seldom exceeded 60 millimicrons, in extracts of cancer tissue they often exceeded 80 millimicrons. The fact that one did not encounter globules as large as those described by Porter and co-workers and also by Gessler and Grey can be explained by the difference of preparation. Both groups of investigators fixed their tissue with osmium tetroxide, which preserved the lipid fraction of the cytoplasmic globules, while in our method lipid linked to the protein was removed by treatment with benzene. The observation of the English authors that extracts of tumors of mice of high cancer strains contain particles measuring only 20 millimicrons is probably also due to the difference in preparation of the extract. Trypsin and a Berkefeld filter will certainly remove all larger globules from the tumor extracts.

COMMENT

While results which my associates and I have obtained with human material confirm the observations of previous workers that larger globules are present in cancer tissue, we hesitate to accept their interpretation that these particles are of extraneous origin and probably virus-like causative agents. Since we⁹ were able to demonstrate very similar spherical bodies in cerebrospinal fluid from persons who had no tumors, we are of the opinion that we are dealing with globular proteins. Those globules under a diameter of 60 millimicrons are apparently normal cell constituents and correspond to the microsomes described by Porter and co-workers¹⁰ in noncancerous animal cells and to the *spherules* observed in the cytoplasm of thrombocytes by Bassis and Bricka.¹¹ The large globules which seem to be characteristic of cancer cells are also very likely globular proteins which have aggregated to large micelles. The question then arises why the globular proteins appear in much larger size in cancer than in benign tissue.

It seems to us that the most plausible explanation is a disturbance in the colloidal state of the cytoplasm of cancer cells. Several physico-chemical and experimental observations point in this direction. Cowdry and Paletta¹² demonstrated by ultracentrifuge studies that cancer cells have a lower viscosity than normal cells. Cramer¹³ found that cancer cells contain relatively more water than normal tissue and that their water content increases directly with the growth rate of the particular tumor as measured by the mitotic index.

9 Hellwig, C. A., Drake, R. L., Voth, H. W., and Bleicher, J. E. *Am J Clin Path* **18** 852, 1948.

10 Porter, K., Claude, A., and Fullam, E. F. *J Exper Med* **81**:233, 1945.

11 Bassis, M., and Bricka, M. *Biochim et biophys acta* **2** 239, 1948.

12 Cowdry, E. V., and Paletta, F. X. *Am J Path* **17** 335, 1941.

13 Cramer, W. J., cited by Cowdry.¹

Also of interest is Spek's¹⁴ experimental observation that the rate of reproduction of *Balantidium* can be increased twenty times by adding lithium chloride to the culture medium, which causes swelling of the cytoplasm of the protozoa. While no definite chemical differences have been demonstrated¹⁵ in the proteins of cancerous and normal cells, marked differences in vital phenomena can apparently be created by different associations and linkages of the cell constituents. The large size of the globular proteins in cancerous cells is very likely an expression of some peculiarity in the colloidal state of the cell.

CONCLUSIONS

Extracts of 23 cancerous and 7 benign human tumors were studied with the electron microscope. Spherical bodies were observed in most tumor extracts, benign as well as cancerous. There was a definite difference in the diameters of these globules, the particles found in cancer extracts being larger than those in benign tissue.

In our opinion these globules are not virus-like causative agents of cancer, but globular proteins. The small spherical bodies are normal cellular constituents, while the larger particles are probably aggregates of the cytoplasmic globules due to an alteration in the colloidal state of the cancer cell.

14 Spek, J, cited by Ostwald, W. *Die Welt der vernachlässigten Dimensionen*, Leipzig, Theodore Steinkopff, 1921, p. 142.

15 Toennis, G. *Cancer Research* **7** 193, 1947.

LEAD POISONING DIAGNOSED BY THE PRESENCE OF NUCLEAR ACID-FAST INCLUSION BODIES IN KIDNEY AND LIVER

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NOT ONLY viruses but other agents may lead to the production of cellular inclusion bodies. Inclusion bodies may occur after the ingestion of certain metallic compounds, e g, lead (Blackman¹) or bismuth (Pappenheimer and Maechling²), and after the injection of aluminum and ferric compounds (Olitsky and Harford³). Inclusion bodies caused by bismuth or lead are acid fast when stained with the Ziehl-Neelsen technic. This property may serve as a useful guide for their further identification.⁴

The finding of inclusion bodies may shed light on the disease underlying an otherwise unexplainable death. The following report of a case elucidates this point.

REPORT OF CASE

A 21 month old boy was admitted to St Catherine's Hospital with a history of increasing irritability of four weeks' duration. Ten hours prior to admission he had vomited profusely. He had always been difficult to manage and was in the habit of placing in his mouth everything that he could put his hands on. He had a normal neonatal period with normal growth and development. On admission he appeared to be dehydrated and was pale, drowsy, irritable and uncooperative. There was a systolic murmur over the mitral region of the heart, the heart rate was 140. The lungs were clear to percussion and auscultation. The abdomen was scaphoid and soft, without abnormal changes. The body temperature was 102 F. The pupils reacted to light, and there was normal accommodation reaction. Flexion of the neck produced some pain. There were a moderately pronounced Brudzinsky sign, an equivocal Babinski sign on the right and a Kernig sign on the right. There was no tenderness of the muscles of the back, and no paresis or paralysis. The cranial nerves were intact. A spinal tap was attempted, but owing to the irritability of the child, only spinal fluid mixed with blood was obtained. The urine showed a specific gravity of 1.020, with acetone (1 plus), 1 to 2 white blood cells and a few epithelial cells. A blood count revealed 2,800,000 red cells. The hemoglobin amounted to 6.3 Gm per hundred cubic centimeters (42.4 per cent). There were 10,000 white blood cells, of which 50 per cent were segmented.

From the Department of Pathology, St Catherine's Hospital

1 Blackman, S S, Jr. Bull Johns Hopkins Hosp **58** 384, 1936

2 Pappenheimer, A M, and Maechling, E H. Am J Path **10** 577, 1934

3 Olitsky, P K, and Harford, C G. Am J Path **13** 729, 1937

4 Wachstein, M. Am J Clin Path **19** 608, 1940

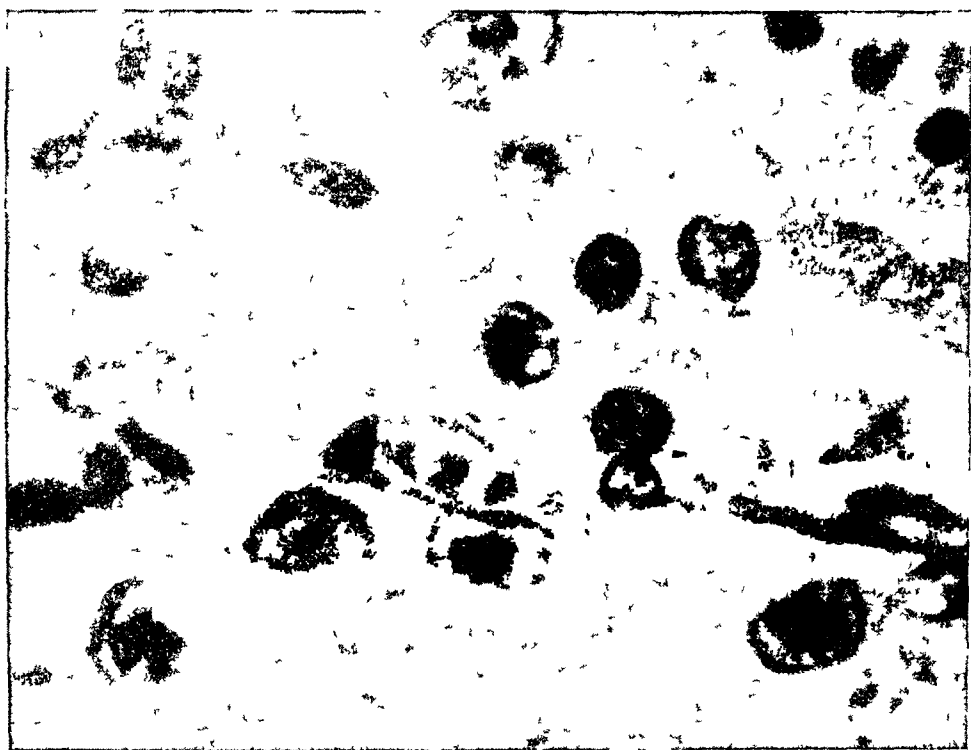
neutrophilic leukocytes, 3 per cent band cells, 40 per cent lymphocytes and 6 per cent monocytes. There was considerable hypochromasia, poikilocytosis, anisocytosis and moderate basophilic stippling of red cells. Five rubricytes were found for 100 white cells. The reticulocyte count was 12 per cent, the thrombocyte count, 270,000. Serum protein was 6.35 Gm per hundred cubic centimeters, with globulin 1.95 Gm and albumin 4.4 Gm. The ascorbic acid level was 1.3 mg per hundred cubic centimeters of plasma. The Kahn reaction of the blood was negative.

The child was given 25 per cent dextrose solution in lactated Ringer's solution U. S. P., but he did not improve. In the following hours spasm of the neck increased. There was tenderness over the posterior region of the thighs. In reaching for water the child seemed to be ataxic. On a second spinal tap, eleven hours after the first, clear fluid under pressure was obtained, containing 25 white cells per cubic millimeter. The total protein was markedly increased to 258 mg per hundred cubic centimeters. Sugar was 100 mg, chlorides 659 mg, per hundred cubic centimeters. A culture of the fluid remained sterile. During the next few hours the condition became progressively worse. The child lapsed into deep coma, spasm of all extremities developed and death followed twenty-four hours after admission.

Necropsy (ten hours after death).—The body was that of a 21 month old white boy in good nutritional state. The mucous membranes and the skin were pale. The abdominal cavity did not contain any increased amount of fluid. The serosal surfaces were smooth and glistening. The pleural cavities contained no excess of free fluid, and there were no adhesions. The heart was slightly enlarged and weighed 80 Gm (normal, 56 Gm). The pericardium was smooth and glistening. The myocardium was flabby and pale. All chambers of the heart were somewhat dilated. The valves were competent. The coronary arteries and the aorta were not unusual. The lungs were heavier than normal and weighed 380 Gm (normal, 155 Gm). On cut section they oozed a fair amount of frothy edema fluid and dark red blood. The liver was enlarged and weighed 600 Gm (normal, 350 Gm). The outer surface was smooth and dark red. On cut section it oozed a fair amount of dark red blood. The structure was indistinct. The spleen was enlarged and weighed 65 Gm (normal, 33 Gm). The cut section was dark purple and congested. Both kidneys were similar and weighed 120 Gm together (normal, 90 Gm). The capsules stripped with ease, revealing a smooth outer surface. The cut section appeared somewhat congested. The proportion between the cortex and the medulla was normal. The pelves, ureters and bladder were not unusual. Cross sections of lumbar and thoracic vertebrae revealed moist, pale, red marrow. The brain was somewhat edematous, and at the base the vessels appeared congested. The meninges did not appear unusual. Coronal sections of the brain did not reveal any significant gross changes.

Microscopic Examination.—Cross section of the heart showed foci of lymphocytic and plasmacytic infiltration as well as occasional proliferation of myocytes. In some areas fibroblastic proliferation with some damage of muscle fibers was noticed. The lungs showed marked edema and congestion. There was also considerable accumulation of lymphocytes and plasmacytes in the walls of the bronchi. The liver showed definite fatty changes and moderate congestion. In preparations stained with sudan III and sudan black, almost each liver cell revealed one or several large fat droplets. Some of the nuclei of the liver cells contained eosinophilic inclusion bodies. These were mostly round and occasionally had a somewhat irregular outline. The nuclei of the liver cells were not significantly changed. The spleen showed considerable increase in the cellularity of Billroth's

cord There was an increase in the numbers of plasmacytes and eosinophilic leukocytes The kidneys on section revealed innumerable nuclear inclusion bodies in the cortex and in the subcortical zone (figure) They occurred in tubules that could be identified as proximal convolutions but also in tubules forming the medullary rays Nearly all these inclusion bodies were eosinophilic in sections stained with hematoxylin-eosin They were round or irregular and varied in size from tiny droplets to some larger than the nuclei One or several inclusion bodies were found in one nucleus Apart from the swelling of the nuclei there were only slight degenerative changes in the cytoplasm of the tubules The inclusion bodies stained orange with the Masson stain as modified by Pollak⁵ and proved to be acid fast No appreciable fat was detected with the sudan stain (sudan III



Section of kidney stained with hematoxylin-eosin Several nuclear inclusion bodies are seen $\times 897$

and sudan black) in the tubular epithelium Paraffin sections were made from various areas of the brain and stained with hematoxylin-eosin In addition, celloidin sections were prepared and stained by the Nissl technic with the aid of Dr H M Zimmerman, Montefiore Hospital, Bronx, N Y The appearance of the meninges was normal There were a few small foci of glial proliferation in the subpial layer of the cortex The remainder of the cortical layers and the subcortex were not remarkable No alteration of blood vessels was seen In the cerebellar sections, a few glial nodules occurred in the molecular layer They were sometimes associated with dropping out of a few nearby Purkinje cells The loss of these cells, while not extensive, was definite and widespread The

5 Pollak, O J Arch Path **37** 294, 1944

blood vessels were normal in appearance. The medulla at the level of the olive revealed no abnormalities. Cross section of other organs did not reveal any other significant changes.

Chemical Test—In 10 Gm composite samples of kidney and liver fixed in formaldehyde solution 15 mg of lead was found in 100 Gm of tissue (highest amount of lead found in control tissue, 0.05 mg per hundred grams) by Dr. A. O. Gettler, Medical Examiner's Office, New York.

Anatomic Diagnosis—Lead poisoning with typical inclusion bodies in kidneys and liver, minimal gliosis and loss of Purkinje cells of the brain, focal subacute myocarditis, congestion and edema of the lungs, congestion of liver, spleen and kidneys, severe fatty changes of the liver, chronic bronchitis.

COMMENT

The case is of special interest because of the nuclear inclusion bodies in the liver and the kidneys which furnished the clue to the explanation of a death that would otherwise have remained obscure. There was nothing in the history of the child that suggested the possibility of lead poisoning. Questioning of the parents, however, after the postmortem diagnosis had been established, revealed the fact that the child was in the habit of putting various objects into his mouth. The child had considerable anemia with increased numbers of reticulocytes and a number of nucleated blood cells in the peripheral circulation. There was also moderate basophilic stippling of red cells. This finding was not considered significant for lead poisoning, since basophilic stippling is not infrequently seen in patients who have anemia due to other causes. The increased number of stippled red cells is of much higher significance in the absence of an appreciable anemia.⁶ A lead line on the gum was not found. A roentgen examination of the long bones was not made, however, no significant changes were seen in the ribs on a routine roentgenogram of the chest.

The spinal fluid was typical for lead poisoning. It must be kept in mind, however, that similar spinal fluid changes are found in other diseases. In lead poisoning a spinal tap yields clear fluid under increased pressure with increased protein content and often pleocytosis.⁷

Although the predominant clinical symptoms were of a cerebral nature, microscopic examination of the brain did not reveal changes which could be considered typical for lead encephalopathy. Lead injury leads to swelling of the vascular epithelium, perivascular and diffuse edema and hyperplastic changes in the leptomeninges as well as to damage of nerve cells and to neuroglial alterations.⁸ It was the opinion

6 Belknap, E. L. J. A. M. A. **139** 818, 1949.

7 Kirchner, E. Deutsche med. Wchnschr. **68** 351, 1942. Bowne, I. B. J. Nat. Med. **36** 187, 1944.

8 Kelatis, A. J. Nerv. & Ment. Dis. **93** 313, 1941.

of Dr H M Zimmerman (chief, Laboratory Division, Montefiore Hospital, Bronx, N Y) that the changes seen in this case were compatible with but by no means characteristic of lead encephalopathy

The microscopic changes in the other organs with the exception of the nuclear inclusion bodies were nonspecific Focal interstitial myocarditis is frequently encountered not only in infectious diseases but in many other pathologic states ⁹

Inclusion bodies such as those seen in this case were first described by Blackman,¹ who found them in each single case at 21 necropsies of children who died of lead poisoning Identical inclusion bodies were repeatedly described in experimental lead poisoning ¹⁰

If proper methods are used, the inclusion bodies can be detected easily They may, however, be overlooked on superficial examination, particularly if, as in the case described here, degenerative changes of the tubular epithelium are scanty This may explain the fact that the presence of inclusion bodies was not referred to in several recent reports of cases of lead poisoning in which postmortem examination had been done

The inclusion bodies stain prominently with Pollak's modification of the Masson stain as suggested by Gorham¹¹ for the detection of distemper inclusion bodies Their acid-fastness furnishes a valuable clue as to their probable nature, since among various inclusion bodies only those caused by lead and bismuth proved to be acid fast ⁴ The morphologic aspect of the bismuth inclusion bodies is, however, so different that their distinction should be made without difficulty The final proof, of course, has to be established by the chemical examination of the tissues for lead

SUMMARY

The cause of death in a 21 month old boy was clarified by the finding of nuclear inclusion bodies in the liver and the kidneys On the basis of their morphologic aspect and acid-fastness the inclusions were assumed to be caused by lead Chemical examination of kidney and liver tissue confirmed this assumption The significance of nuclear inclusion bodies for the diagnosis of lead poisoning is stressed

⁹ Saphir, O Arch Path **32** 1000, 1942, **33** 88, 1942 Fawcett, R M *ibid* **45** 25, 1948

¹⁰ Finner, L L, and Calvery, H Arch Path **27** 433, 1939 Diaz-Rivera, R S, and Horn, R C, Jr Proc Exper Biol & Med **59** 161, 1945 Dalldorf, G, and Williams, R R Science **102** 668, 1945 Wachstein ⁴

¹¹ Gorham, J R Science **107** 175, 1947

HISTOGENESIS OF BASAL CELL CARCINOMA

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AND
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A PREVIOUS study of epithelial tumors of the skin was made during the ten year period from January 1939 to January 1949, which included 984 such tumors. During the course of the study 182 tumors diagnosed as basal cell carcinoma were included in the series. Because conclusions were reached which seemed to be incompatible with those expressed in recent studies of the histogenesis of the basal cell carcinoma,¹ it was deemed advisable to carry on a further study of this type of tumor to determine whether or not present concepts of the histogenesis of the basal cell carcinoma are valid. The conclusions derived from this study corroborated the older concepts first suggested by Krompecher.

HISTORICAL REVIEW

Basal cell carcinoma of the skin was first described by Jacob, in 1827, whence the eponym jacobian ulcer was derived. It was recognized as a slowly growing, invasive, ulcerating but nonmetastasizing tumor of the skin. However, an accurate pathologic study of this tumor was not made until 1900, when Krompecher² described 21 cases and attempted to group them according to four types of growth. This classification is of interest as it forms the basis of most subsequently suggested arrangements and is as valid at the present time as when it was suggested. Krompecher divided them into (1) cases of a solid pouchlike downgrowth of epithelium, (2) cases of an epithelial mass containing cysts, (3) cases of a glandular form made up of interlacing strands of epithelial cells and (4) cases in which nests of epithelium formed parakeratotic pearls.

However, in 1902 Krompecher expanded his concept of basal cell carcinoma to include tumors occurring in the mucous membranes of mouth, nose, pharynx, larynx, esophagus and vagina. Histologists

From the Department of Pathology of Passavant Memorial Hospital and Northwestern University Medical School

1 Foot, N C. Am J Path **23** 1, 1947

2 Krompecher, E. Beitr z path Anat u z allg Path **28** 1, 1900

now know that while these tumors are histologically similar to basal cell carcinoma they are biologically more malignant, being undifferentiated squamous cell carcinoma, and should not be included in the category of basal cell carcinoma

Mallory³ demonstrated fine longitudinal epithelial fibers in basal cell carcinoma, which he believed were identical with those found in embryonic hair follicles. He therefore concluded that this type of carcinoma arose from the hair matrix, including the sebaceous and sweat glands. This work, however, received little attention, and the theory prevailed that the basal epithelium of the epidermis was the site of origin of the basal cell carcinoma.

Paul,⁴ in his observations on the origin, cause and treatment of rodent ulcer in Australia, adhered closely to the original concept of Krompecher and suggested four types: (1) a reticular type, in which the growth is composed largely of strands of cells enclosing a stroma, a growth in which cystic structures may be formed, (2) a budding type, in which a solid type of growth occurs, showing budding or finger-like projections, (3) a combined reticular and budding type, (4) a basal cell type. Paul expressed the belief that the first three types represented growths whose origin lies in the pilosebaceous apparatus, the normal site of which is in the corium, and are the least malignant of the types named. The fourth type was due to a proliferation of the epidermis. He stressed the effect of actinic rays in causing rodent ulcer.

Haythorn⁵ made the first careful study of the histogenesis of the so-called basal cell carcinoma. In a study of 139 specimens, he found that all conformed to the characteristics suggested in Krompecher's classification and that all varieties were included. It was unusual to find a single tumor which did not include more than one of Krompecher's types. In an exhaustive study of the staining characteristics of the tumor cells and of the basement membrane, he reached the conclusion that they all originate from hair matrix. Thus, there are commonly found more or less perfectly formed hair shafts in the basal cell tumor. Silver staining technics show the type of basement membrane to be similar to that around the hair follicle. The distribution of pigment in a basal cell carcinoma he considered as equivocal evidence of the tumor's origin. He expressed the belief that sebaceous glands frequently form basal cell tumors but that sweat glands do not take part. The apparent continuity of the tumor masses with the basal layer of the epidermis, he concluded, was only apparent.

3 Mallory, F. B. *J. A. M. A.* **55** 1513, 1910

4 Paul, N. M. *J. Australia* **1** 85, 1923

5 Haythorn, S. R. *Am. J. Cancer* **15** 1969, 1931

The difficulty of accepting the basal layer origin of these tumors was due primarily to the concept of differentiation of the basal layer of cells. The normal basal layer, when it differentiates, forms large prickly cells which are totally unlike the basal carcinoma cells. Krompecher claimed that the cells of the tumor were basal cells which had retained their embryonic structure and did not differentiate like skin, and therefore did not take the form of prickly cells. Haythorn expressed the opinion that if this were so, then the basal cell carcinoma, being derived from embryonic cells, should retain the characteristics of other tumors derived from embryonic cells, that is, they should be highly malignant. This is not true, however, the basal cell carcinoma being cancerous to only a low degree. Haythorn attempted to explain this discrepancy by deriving it from the hair matrix, in which location differentiation is away from the formation of prickles and toward the formation of longitudinal fibers. He therefore derived basal cell carcinoma from the pilosebaceous apparatus, rejecting any evidence that the tumor tissue is continuous with the basal layer of the epidermis as being only apparent.

At the time that Haythorn's work appeared in print, Niles⁶ published a report of metastases derived from a basal cell carcinoma. This report is mentioned only to say that the illustrations are typical of a squamous cell type of growth and must be rejected. Numerous reports of metastasizing basal cell carcinoma have been published,⁷ but none of them pass the test of close investigation.

Montgomery,⁸ in a study of the histogenesis of the basal cell carcinoma, grouped the tumors according to two forms: (1) the benign form (epithelioma adenoides cysticum and cylindroma), which may arise from multiple points of origin, that is, from the basal cells of the epidermis, from the basal cells of the outer sheath of the hair follicle, from the sebaceous gland and the sweat gland, (2) the malignant form, which may rarely arise from the hair matrix, more often from the basal cells of the outer root sheath of the hair follicle but most often from single or multiple points of origin in the basal layer of the epidermis. Montgomery determined that silver stains were not of value in distinguishing a tumor of basal cell origin from a tumor arising in a hair matrix. Melanin formation likewise did not indicate the origin of the basal cell growth. He likewise concluded that the occurrence of a mixed basal cell-squamous cell carcinoma represents

6 Niles, H. D. *Am J Cancer* **15** 2341, 1931.

7 Finnerud, C. W. *J A M A* **82** 775, 1924. De Navasquez, S. *J Path & Bact* **53** 437, 1941. Spies, J. W. *Arch Surg* **21** 365, 1930. Small, C. S., and Hankins, F. D. *Arch Path* **47** 196, 1949.

8 Montgomery, H. *Radiology* **25** 8, 1935.

a metamorphosis from basal to squamous cells and decried the concept of a fundamental and separate histogenesis

About this time a theory of the histogenesis of basal cell carcinoma was suggested by Glasunow⁹ and supported by McFarland, Ciccone and Gelehrte¹⁰ In an investigation of 254 cases of basal cell carcinoma of the face, Glasunow was convinced that the distribution of the lesions so closely corresponded with the position of the "facial fissures," or intervals between the various embryonal fissures or buds, through whose final concrescence the face is formed, as to prove that the tumors are of embryonal origin, that is, dysontogenetic He called them facial fissure carcinoids or skin carcinoids However, he was unable to explain the genesis of tumors found in locations other than the face

Warren, Gates and Butterfield¹¹ studied 321 cases in an attempt to correlate the histologic type with clinical behavior They divided the series into five types (1) basal cell carcinoma, (2) basal cell carcinoma with foci of keratinization, (3) mixed "basosquamous" carcinoma, (4) hair matrix carcinoma, (5) cystic basal cell carcinoma In their study they frequently found the tumor to be multicentric in origin, growing from several distinct points of the overlying epidermis and occasionally from skin appendages The cystic type they held to be the result of degeneration of either tumor or stroma In their study of five year cures following irradiation, the percentages given are type 1, 41 per cent, type 2, 20 per cent, type 4, 33 per cent, type 5, 100 per cent They concluded that development toward a basal cell or a hair matrix tumor denotes low malignancy, while differentiation toward a squamous cell carcinoma is present in the more dangerous tumors

Gate, Massia and Delbos,¹² in a discussion of pigmented basal cell epithelioma, cited the incontestability (in their opinion) of the theory that the basal cell tumor takes origin from the pilar structures, the sebaceous glands and sometimes even the sudoriferous glands They stated that the hypothesis establishing a relationship between the basal cells of the epidermis and the cells of the basal cell carcinoma ought to be abandoned They found an incidence of pigmented forms of 6 to 10 per cent These have the same prognosis as the ordinary type, and the course and the mode of therapy are not altered The authors stressed the importance of differential diagnosis respecting these tumors

9 Glasunow, M *Frankfurt Ztschr f Path* **46** 140, 1933

10 McFarland, J Ciccone, E F, and Gelehrte, J *Am J Cancer* **25** 273, 1935

11 Warren, S, Gates, O, and Butterfield, P W *New England J Med* **215** 1060, 1936

12 Gate, J, Massia, G, and Delbos, J *Ann de dermat et syph* **8** 337, 1937

and cancerous melanoma, a tumor which carries a much graver prognosis

Holtzman and Bolker¹³ suggested an interesting and unusual theory to explain the peculiar biologic behavior of the basal cell carcinoma. They fostered the concept of the basal layer as being a continuous protoplasmic mass containing nuclear elements. This continuity diminishes as the prickle layer is reached. By microdissection, the prickle cells can be separated with ease, while the basal cells can be separated only with difficulty. In the basal cell carcinoma the same continuity of protoplasm exists, retains connection with the point of origin of the epidermis, as proved by serial sections, and explains lack of metastases. Although attractive, this theory is untenable since intercellular bridges may be present in the basal layer, according to most authorities, and can frequently be demonstrated in the cells of the basal cell carcinoma. These authors explain the formation of basal cell carcinoma by stating that the cells proliferate in one direction corresponding to the polarity of the ancestral basal cell at the time it underwent mutation.

Krompecher, in his original work, included so-called basal cell carcinoma of the oral, laryngeal, pharyngeal, nasal, esophageal and vaginal mucous membranes. Owen¹⁴ made a thorough study of 836 lesions of mucous membranes diagnosed as carcinoma to determine the incidence of basal cell growths in these areas. None were found. The growths usually mistaken for basal cell carcinoma are pathologically and clinically highly invasive squamous cell carcinoma. At the present time the exclusively cutaneous origin of the basal cell carcinoma is accepted by practically everyone.

The recent work of Foot¹ has redirected attention to the pilosebaceous apparatus as the point of origin of the basal cell carcinoma. Foot devised an elaborate system of classification of these tumors depending on the points of origin of the growths.

1 Pilar type, derived from the hair matrix. The overlying epidermis is intact, atrophic and, in the late stages, ulcerated.

- (a) Pilar type proper, imitating the structure of the hair follicles
- (b) Primordial type, forming large spherical masses of cells
- (c) Cylindric cell or ribbon type, rarely encountered
- (d) Cystic type, a variant of *a* and *b*

2 Sudoriparous glandular type

- (a) Adenoid type
- (b) Hydradenomatous type, either resembling the solid hydradenoma or the papillary cystic hydradenoma

3 Basal cell type, uncommon. Foot considered this type to be more properly a plexiform epidermoid carcinoma.

13 Holtzman, I. N., and Bolker, H. *Am. J. Roentgenol.* **47**: 463, 1942.

14 Owen, M. *Arch. Path.* **10**: 386, 1930.

With the technic of impregnating neurofibrils with silver (Nonidez' modification of the Ramón y Cajal method) Foot demonstrated plexuses of prominent and readily visible nerve filaments in the pilar papillae and about the sebaceous glands. Very few such fibrils were demonstrated in the papillae of the normal dermis or in the basal layers of the epidermis. In adnexal carcinoma, there are heavy bundles of nonmyelinated fibers running in the stroma. This the author interpreted as an implication that the adnexal carcinoma was related to hairs, sebaceous glands and sudoriparous glands rather than to basal elements of the epidermis. Foot reiterated the theory of the hair matrix origin of the basal cell carcinoma suggested by Haythorn and agreed with the latter in almost every particular. He also insisted that the contact of masses of basal cells with the basal layer of the epidermis is only apparent and demonstrated this to his own satisfaction by means of serial sections of early basal cell lesions and construction of wax models. He denied the basal cell origin of the lesion and suggested the name originally introduced by Haythorn, "adnexal carcinoma," to include all forms of basal cell carcinoma.

Willis¹⁵ insisted on the multicentric origin of basal cell carcinomas, from the epidermis itself, from the pilosebaceous apparatus and from the sweat glands, either singly or in combination. He used a simple classification: superficial basal cell carcinoma, derived from the basal layer of the epithelium, and subepidermal basal cell carcinoma, derived from the adnexae. In his own words:

Studies of early superficial basal cell tumors show unmistakably that these often arise, not from single minute foci, but from multiple foci in considerable areas of epidermis, and that the basal cells of hair follicles and shafts and of the skin glands may also participate in the cancerous change.

In a discussion of the presence of nerves he expressed the belief that there is no convincing evidence that cancer cells are innervated, that all genuine nerves observed in tumors are inclusions and the supposed nerve endings in tumor cells are only retraction bulbs or varicose nerve fibers contiguous with the cells. Nerves show a remarkable persistence in the substance of invading tumors so that all relationships of residual nerve fibers and tumor elements will be demonstrated. In addition, when a nerve trunk is damaged by a tumor, numerous new fibrils grow from the lining axis-cylinders of the proximal stump of the nerve trunk. These new axis-cylinders not only grow within the perineural tube of the proximal portion but penetrate into the neoplastic tissue. If this concept is valid, the fact that nerve fibers are present in the masses of basal cells as demonstrated by Foot is no particular indication that these cells originate from the pilar

15 Willis, R. A. *Pathology of Tumors*, St. Louis, C. V. Mosby Company, 1947.

papillae and sebaceous glands. Their presence merely indicates the fortuitous circumstances that proliferating neurofibrils of the surrounding damaged or irritated nerve bundles have infiltrated the tumor.

The generally accepted nonspecificity of the silver impregnation method of demonstrating nerve fibers is likewise another factor in weakening the validity of the work of Foot.

MATERIAL AND METHODS

In the present study there are 182 examples of the basal cell type of carcinoma, occurring in 166 patients. There was 1 patient with two simultaneously occurring lesions of this type. Fifteen of the specimens represented recurrences. Three patients had a coexistent but distinct squamous cell carcinoma. The tumors are first tabulated as to general histologic type (table 1). Of the total number of epithelial tumors of the skin studied, 18.5 per cent represented basal cell carcinoma.

The classic description of the basal cell carcinoma invariably includes its distribution as being preponderantly localized to an area of the face bounded by the hair line, the ears and the upper lip. That it may occur on almost every part of the body is readily seen in table 2. Assuming that those lesions listed

TABLE 1—*Tumors Grouped According to Histologic Types*

	Number	Percentage
Basal cell carcinoma	182	100
"Basal cell" type	150	82.4
Mixed basosquamous type	25	13.7
Pigmented basal cell type	6	3.2
Mixed pigmented basosquamous type	1	0.7

under "face" and "unclassified" are present in the classic location, 31, or 17 per cent, of the total are present in another part of the body. The importance of this atypical distribution has rarely been emphasized in any large series.

In an attempt to prove the worth of Foot's classification of adnexal carcinoma, the 182 examples were carefully studied and tabulated (table 3). It was evident from the beginning that many of the specimens contained elements of several different types and that it was unusual for any single one to be listed under any single type. Although classification according to a number of different types based on histologic characteristics is valid in a descriptive way, the impression was gained that it added little to the final understanding of the histogenesis of the tumor, and was cumbersome.

In a preliminary survey of the material, sections stained with hematoxylin and eosin were examined, and the tumors were classified. Fifty-three, or 29.1 per cent, showed histologic evidence of origin from or continuity with the basal layer of the epidermis, either with or without evidence of simultaneous origin from appendages of the skin. This figure is undoubtedly low, since in many cases, if definite evidence of origin from the basal layer was not present in an ulcerated or advanced lesion, the tumor was placed in another category. Several early lesions had multicentric origins in both the basal layer and the pilosebaceous apparatus. In 1 case there was found incidentally in a section of skin removed for a squamous cell carcinoma a very small basal cell carcinoma, distinct from the squamous cell tumor. It showed definite evidence of originating from the basal layers.

TABLE 2—*Distribution of Specimens of Different Types of Basal Cell Carcinoma**

Location	Basal	Mixed	Pigmented	Pigmented Mixed
Temple	4			
Forehead	12	1	1	
Eyebrow	1			
Eyelid	23	3	1	
Ear	6	5		
Cheek	13	3		
Nose	22	4		
Chin	4			
Neck	7			1
Scalp	2	1		
Lower jaw	2			
Upper lip	12	1		
"Face"	22	6	1	
Thoracic wall	1		1	
Nipple of breast	1			
Back	6			
Upper arm	2			
Wrist	1			
Dorsum of hand	3			
Inguinal region	1			
Thigh	2			
Unclassified	4	1	2	

* This tabulation is based on a total of 182 specimens obtained from 166 patients. Fifteen specimens represented recurrences. There was 1 patient from whom 2 specimens of basal cell carcinoma were simultaneously removed. There were 3 patients with coexistent basal and squamous cell carcinoma.

TABLE 3—*Classification of Specimens of Basal Cell Carcinoma According to the Classification of Foot*

Type	Basal	Basosquamous	Pigmented	Pigmented Mixed
Basal	7	1	3	
Primordial	43	9	2	
Pilar	5	1		
Cylindric	2			
Cystic	6			
Adenoid	4			
Hydradenomatous	4			
Primordial cystic	12	1		
Primordial pilar	4	1		
Primordial basal	24	5		1
Primordial adenoid cystic	1			
Primordial cylindric	3	1		
Primordial basal cystic	7	2	1	
Pilar basal	6	1		
Cystic basal	3			
Basal cylindric	1			
Basal adenoid	1			
Primordial pilar-cystic	2	1		
Primordial pilar cystic basal	2			
Pilar adenoid		1		
Primordial adenoid	5			
Primordial pilar basal	2			
Cystic cylindric	2			
Primordial pilar adenoid	2	1		
Pilar cylindric	2			

Specimens which were not ulcerated or which contained a definite inflammatory reaction were then selected for further study. These were stained with Masson's trichrome stain. Suitable small or histologically well preserved specimens were studied further by means of Wright's silver impregnation method. A few very small ones were selected for study by means of serial sections.

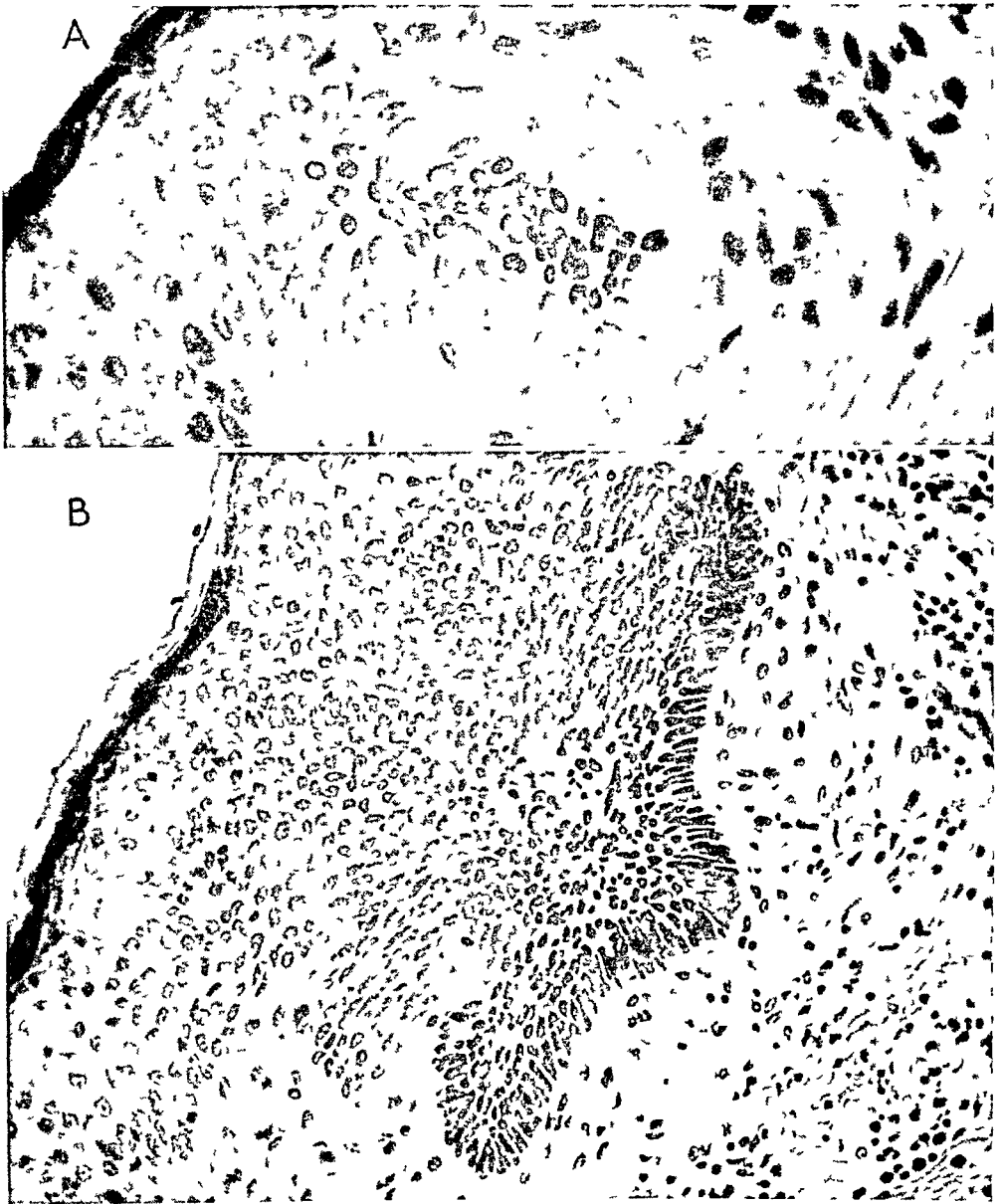


Fig 1—*A*, small satellite basal cell lesion extending for 100 microns. It originates from the basal layer of the epidermis. Case 10870, hematoxylin and eosin stain, $\times 260$. *B*, satellite lesion showing gradual transition from the prickle cell layer to the mass of basal cells. The basement membrane is intact. Case 12803, hematoxylin and eosin stain, $\times 260$.

RESULTS

The first and most striking characteristic of the basal cell carcinoma was its multicentric origin. In the great majority of specimens examined, with the pos-

sible exception of the very small ones, the multicentric origin was seen, the tumor cells arising from the sheath of the pilar apparatus, from the basal layer of the epidermis, occasionally from sebaceous glands and rarely from sudoriferous glands. This multicentric origin could be seen in the form of a tumor having isolated and independent points of origin in the basal layer, or arising from independent and separate pilar structures, or presenting any combination of these two. In one early lesion studied serially, although it was originally classi-

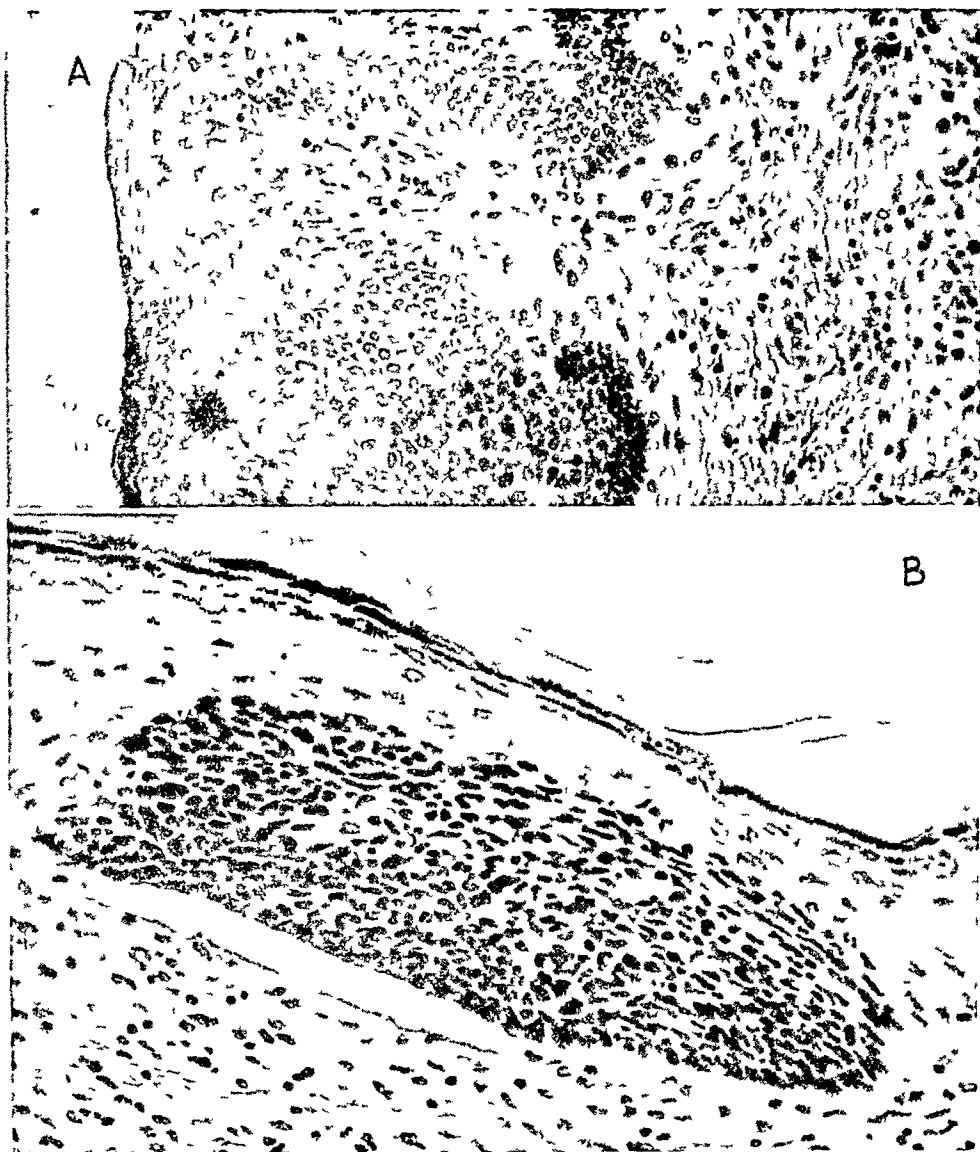


Fig 2—*A*, lesions similar to that in figure 1*B*. Serial sections showed absence of contact with hair follicles. Case 12803, hematoxylin and eosin stain, $\times 120$. *B*, abrupt transition from the prickle cell layer to the mass of basal cells. The basement membrane is intact. The section contains no hair follicles but shows multiple similar foci of origin. Case 12803, Masson trichrome stain, $\times 240$.

fied on preliminary examination as a tumor of the pilar type, small satellite foci originating from the basal layer were observed (case 10870). Another tumor on serial examination was found to have multiple widely separated and independent points of origin in the basal layer as well as multiple points of origin in the pilar structures (case 12803).

Foot¹ and Haythorn⁴ denied that the masses of tumor cells were in any way continuous with the epidermis, explaining such a relationship as being only



Fig 3—*A*, masses of tumor cells originating from the outer sheath of the hair follicle. Masson trichrome stain, $\times 180$. *B*, focus of basal cell carcinoma originating from the outer sheath of the superficially located one third of a hair follicle. Hematoxylin and eosin stain, $\times 240$.

apparent. This was not borne out in our studies. In the preliminary examination of hematoxylin-eosin preparations, the specimens were classified with reference to basal origin. Growths of this origin formed 29.1 per cent of the total number. Many of those which exhibited a basal origin were then examined with Masson's trichrome stain. It was established that cellular continuity was

actually present between the basal cell masses and the basal portions of the epidermis. Usually there was a rather abrupt transition between the two types of cells, but occasionally a gradual transition occurred. The basement membranes of the basal layer of the epidermis and the basal cell masses were continuous and uninterrupted. This was in marked contrast to the type of lesion in which upward expanding masses of basal cells actually encroached on the epidermis.

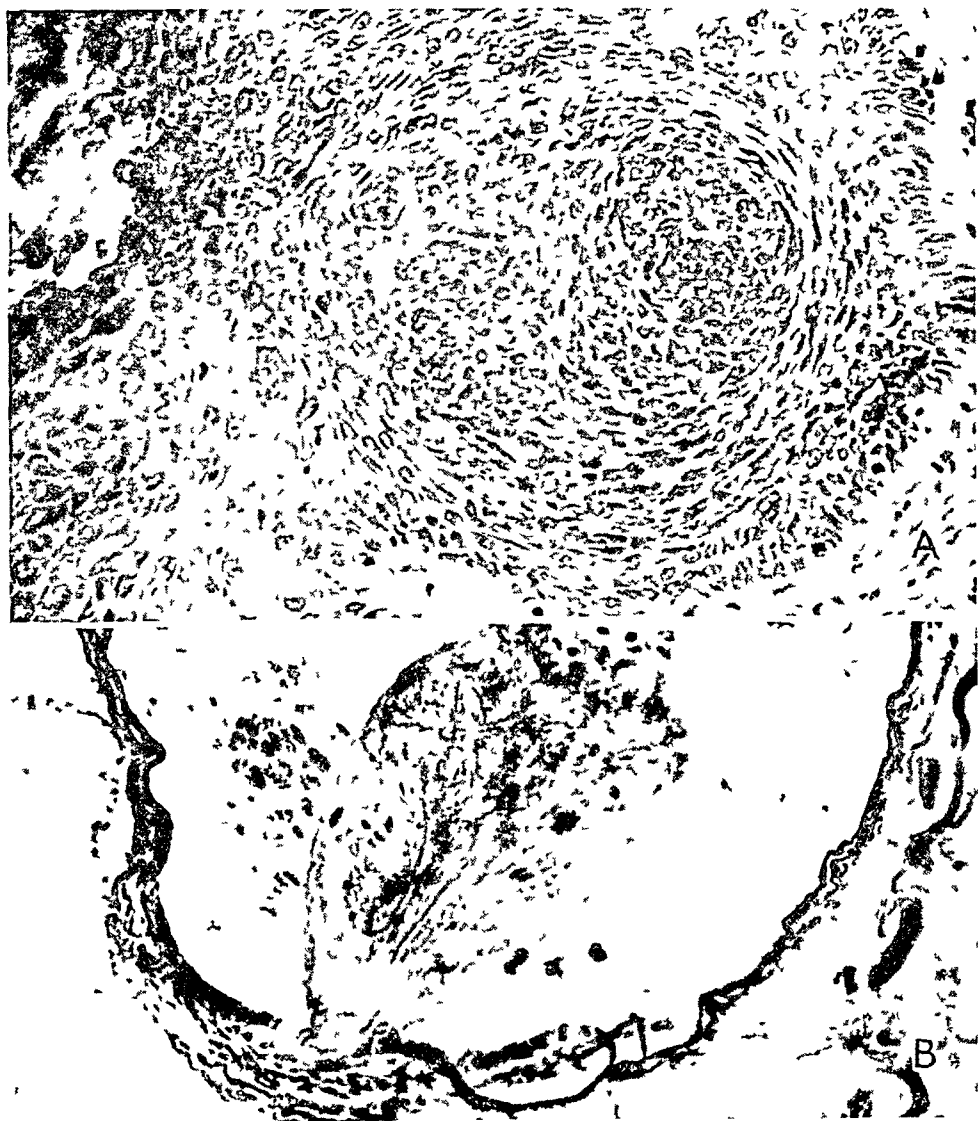


Fig 4—*A*, small intraepidermal focus of basal cell carcinoma found incidentally in an examination of squamous cell carcinoma, but independent of the latter, grade 1. Hematoxylin and eosin stain, $\times 120$. *B*, hair matrix showing neurofibrils. Silver impregnation, $\times 240$.

(subepithelial type of Willis). In the latter situation there was a very fine but recognizable layer of collagenous fibers between the upwardly expanding neoplastic tissue and the epidermis. The epidermis likewise was thin and appeared locally compressed.



Fig 5—A, hair matrix showing neurofibrils Silver impregnation, $\times 240$
 B, hair matrix showing neurofibrils, Silver impregnation, $\times 240$ C, coarse
 neurofibrils in a focus of basal cell carcinoma Note the haphazard distribution
 Silver impregnation, $\times 240$

At this point it was decided that little or nothing new of value would be gained by a study of moderately small lesions. However, if the multicentric origin of the tumor was valid, a study of small satellite points of origin at the periphery of the lesion might give additional information. This proved to be true, and definite origin from the sheath of the pilar apparatus and basal layer was demonstrated in multiple small surrounding satellite foci. Several specimens were sectioned serially with interesting results.

Case 9707 There was multicentric origin from the external sheaths of the superficial one third of the hair shafts and from the basal layer of the epidermis.

Case 10870 This case was chosen as an instance of the pure pilar or sub-epithelial type. On serial section, there were multiple small foci of origin in the hair matrix, the upper portion of the sheath of the hair shaft and sebaceous glands and also multiple small foci of origin in the basal layer of the epidermis. One of the latter extended to a width of approximately 100 microns.

Case 12803 The specimen was a moderately large slightly ulcerated basal cell carcinoma. Serial study revealed multiple small foci of origin in the basal layer of the epidermis and in the sheath of the upper portion of the hair follicle. The deeper lying masses contained rudimentary hair follicles and were obviously pilar in origin. Three different sections of this tumor were studied serially, with identical results in each instance.

Case 14742 Serial study of the tumor showed multiple independent foci of origin in the basal layer and in the hair follicles.

Suitable specimens were studied by means of Wright's technic of silver impregnation. The fine neurofibrils in the pilar matrix were easily demonstrated in many sections. However, demonstrating similar fibrils in the neoplastic masses was much more difficult. In the majority of specimens these could not be demonstrated, and when present, they were distributed unevenly and haphazardly, so as to suggest a fortuitous occurrence.

COMMENT

It would not be incompatible with present day knowledge of the genesis of carcinoma to assume that there is a multicentric origin of the basal cell carcinoma in the basal layer of the epidermis, the sheaths of hair follicles and sebaceous glands. Morphologically the results of the present study tended to bear out this concept. The evidence that carcinoma of this type originates from the sudoriferous glands is much more inconclusive and a definite statement concerning this cannot be made on the basis of the present study. Morphologic evidence that the carcinoma originates from the hair matrix was lacking in most sections and the statement that it has such an origin must be based on inference.

Theories of histogenesis based on analogy are fraught with danger and must be accepted with reserve. That neurofibrils were present in the basal cell masses could easily be shown in some specimens. However, if their presence is to be used as a basis for proof of the pilar origin of the basal cell carcinoma, they must be demonstrated in all or at least in the majority of specimens. This could not be done in the present series. Studies of sections containing neurofibrils showed

an irregular and unorganized distribution, suggesting a fortuitous presence. This was emphasized by Willis, who explained that there was no convincing evidence that neoplastic tissue was innervated, that all relationships between residual nerve fibers and tumor elements could be demonstrated and that the presence of nerve fibrils indicated that a nerve trunk had been damaged by the tumor and that numerous fibrils were growing into the neoplastic tissue.

That the contact between masses of basal cells and the basal layer of the epidermis is only apparent was not borne out in the present study. Serial sections of small satellite lesions proved conclusively that the neoplastic tissue originated solely from the basal layer without any possibility of contact with adnexal structures. Histologically, the differentiation between a neoplasm having an actual origin in the basal layer and an underlying expansile mass of neoplastic cells encroaching on the basal layer was relatively simple. This was true of hematoxylin and eosin preparations as well as of those stained with Masson's trichrome stain. The lack of disruption between the basement membrane of the basal layer and that of the tumor masses corroborates this point.

An interesting study was carried out by Hoffman¹⁶. In a statistical analysis of the rates of growth of the cells of various strata of the epidermis, he found that the ratio of the rate of growth of spinous cells to that of basal cells is 3:1. This is similar to the ratio of the rates of growth of squamous cell carcinoma and basal cell carcinoma, which is 3.6:1. Although purely inferential, this points to the basal type of cell as the progenitor of the basal cell carcinoma.

In conclusion one may say that the study of the present series of 182 specimens of basal cell carcinoma of the skin supports the concept of basal cell carcinoma as having multicentric points of origin in (a) the basal layer of the epidermis, (b) the sheaths of the hair follicles, (c) the pilar papillae, and (d) occasionally the sebaceous glands. A given tumor may arise from any one or from all of these structures. Definite evidence that basal cell carcinoma may originate also from the sweat glands was not found in the present series.

SUMMARY

The history of the development of knowledge of the basal cell carcinoma is reviewed, and the various theories of the histogenesis of this type of carcinoma are discussed and analyzed.

A study of 182 specimens of basal cell carcinoma is presented. The theory that basal cell carcinoma has a multicentric origin in the basal layer of the epidermis and/or in the pilosebaceous apparatus is supported. The evidence that it may arise also from the sudoriferous glands appears inconclusive.

NONLIPID RETICULOENDOTHELIOSIS LETTERER-SIWE DISEASE

Report of a Case

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ABOUT twenty-five years ago Letterer¹ reported splenohepatomegaly associated with anemia and a purpuric eruption which had occurred in an infant that died four days after being admitted to the hospital. Necropsy revealed marked proliferation of the reticuloendothelial cells, which were invading and replacing the normal structures of the liver, spleen, lymph nodes, bone marrow and skin. A similar case was reported by Siwe² in 1933, at which time he grouped his case with previously reported cases³ as instances of what he believed to be a clinicopathologic entity. The criteria of this syndrome included a nonfamilial, nonhereditary disease of infants of unknown cause and fatal terminus, with an acute onset, hemorrhagic tendencies, secondary progressive anemia, lymphadenopathy, splenomegaly, hepatomegaly and localized tumors of bone. The morphologic changes consisted of generalized hyperplasia of the reticuloendothelial system with characteristic large mononuclear cells which were not lipid storing. Abt and Denenholz⁴ chose the title "Letterer-Siwe's Disease" to cover this disease process.

Because the number of cases up to last year (1948) which met the original established criteria for this disease did not exceed 24,⁵ and because of the present lack of uniformity in the correlation of the so-called reticuloendothelioses, it was deemed justifiable to report another case which falls in this rare category and which might aid in the future clarification and classification of the cases of disease of the reticuloendothelial system.

REPORT OF CASE

An 11 month old white girl was admitted to Temple University Hospital in a serious condition. She was born, Jan 18, 1948, as a full term, spontaneous vertex delivery with a labor of seventeen hours. The birth weight was 8 pounds 4 ounces.

From the Department of Pathology, Temple University School of Medicine

1 Letterer, E. Frankfurt Ztschr f Path **30** 377, 1924

2 Siwe, S. Ztschr f Kinderh **55** 212, 1933

3 (a) Letterer¹ (b) Akiba, R. Virchows Arch f path Anat **260** 262, 1926

(c) Guizetti, H. U. ibid **282** 194, 1931

4 Abt, A. F., and Denenholz, E. J. Am J Dis Child **51** 499, 1936

5 Schafer, E. L. Am J Path **25** 49, 1949

(37 Kg) The mother, the father and a brother, 3 years of age, were in normal health. The child was immunized for diphtheria and pertussis in September and October 1948 and for smallpox in November 1948. Her development was considered normal, and she had no previous illnesses or hospitalizations.

The infant was in apparently good health until about three weeks prior to admission, at which time there was an onset of a "throat infection," accompanied by a slight cough and a rectal temperature of 104 F. Treatment consisted of oral administration of sulfonamides, with abatement of symptoms in three days.

Nothing unusual was then observed until ten days prior to admission, at which time it was noted that the child became listless. A slight nonproductive cough developed, accompanied by a low grade fever. Her temperature returned to normal after two days. This was followed by mild postprandial vomiting, which also subsided in two days. On December 13, six days prior to admission, an eruption was observed in the vulvar area which was diagnosed as a diaper rash. This "rash" gradually spread to involve the trunk, the face and all the extremities. In addition, vomiting again became manifest, and on the day prior to admission the child regurgitated all of her feedings. A few hours before she was brought to the hospital obvious dyspnea became apparent. Her condition became grave, and she was admitted to the hospital for treatment on December 19.

Examination revealed a well developed, well nourished white girl who was obviously seriously ill. The rectal temperature was 95 F. A marked pallor and multiple petechiae were noted over the face, scalp, trunk, upper arms, labia and about the anus. A few shotty left posterior cervical nodes were palpable. A blowing systolic murmur was audible at the left second interspace. The liver was palpable 3 cm below the costal cage on the right, and the spleen was palpable 4 cm below the rib cage on the left. Both were firm and smooth. The extremities were flaccid, and there was mild pitting edema of the legs.

An intern who made a blood count on the evening of her admission reported 7.5 Gm of hemoglobin, 1,250,000 erythrocytes and 41,100 leukocytes with many "blast" forms. On the basis of the clinical and laboratory findings a tentative diagnosis of acute leukemia was made, and treatment was begun immediately. This included transfusion of whole blood (500 cc), administration of penicillin, aminopterin (4-aminopteroylglutamic acid) (1 mg) and crude liver extract, and supportive oxygen therapy. The following morning the hematologic studies were repeated, and a biopsy of tibial marrow was made. The peripheral blood revealed 7.2 Gm of hemoglobin, 2,540,000 erythrocytes, 5,900 leukocytes, with neutrophilic myelocytes 73 per cent, lymphocytes 23 per cent and monocytes 4 per cent, 5,100 thrombocytes and 63 nucleated erythrocytes per hundred leukocytes. (The latter were probably misinterpreted in the original examination made by the intern.) Slight bleeding from small fissures and ulceration of the lips and mouth occurred in addition to minimal hemorrhages of the nose and the vagina. The stools were tarry. A low grade fever made its appearance and persisted for the remainder of the patient's illness. The biopsy of the marrow revealed neutrophilic, segmented cells of the granulocytic series, 83 per cent, eosinophilic cells of this series, 0.7 per cent, progranulocytes, 3.3 per cent, mitotic figures, 1.0 per cent, lymphocytes, 6.3 per cent, nucleated erythrocytes (rubricytes), 66.0 per cent, prorubricytes, 10.3 per cent, rubriblasts, 4.0 per cent, erythroid-myeloid ratio, 80.3:19.7 or 1:0.2. Because of the findings and the enormous increase in nucleated red cells in the peripheral blood, the impression was that of an "erythroblastic hyperplasia (anemia) associated with hemorrhage, leukopenia and thrombopenia."

An additional transfusion of 500 cc of whole blood was given on December 23. Examination of the eyegrounds revealed only extreme generalized pallor. The

following day a test of the epinephrine response⁶ was made, which showed a slight rise in the circulating leukocytes. It was felt at this time that the picture fitted "Doan's splenic panhematopenia syndrome"⁶. However, because of the increasing gravity of the child's condition and because of some response as measured by the epinephrine test, the spleen was removed on December 24, with the patient under open drop ether anesthesia. Three hundred cubic centimeters of whole blood was given during the operation.

The removed spleen measured 10 by 7 by 3.5 cm and weighed 120 Gm (normal, 25 Gm). The external surface was not remarkable. The parenchyma was firm, no follicles were demonstrable, and the cut surfaces showed multiple dark areas suggesting hemorrhage. At one pole there were several small irregular white areas which had an appearance not unlike tubercles. Microscopic examination showed recent areas of hemorrhage and necrosis. In other fields multiple irregular areas of infarction were present. The sinusoids were greatly distended, and in many there were large young cells resembling histiocytes. The follicular pattern was completely effaced by the aforementioned changes, and one of the most interesting features noted was multiple areas of hemopoiesis, which was presumed to represent myeloid metaplasia. The changes were considered so nonspecific that a definitive diagnosis could not be made.

The postoperative course continued to be relatively good, and it was felt that the general condition of the patient was better than prior to splenectomy. On December 28 the coagulation time was reported as 2 minutes 50 seconds and the bleeding time as 1 minute. On the following day the child's temperature was 103 F, the highest recorded since her admission. The cause of the febrile course continued to remain obscure. Laboratory studies now revealed a hemoglobin level of 9.7 Gm with erythrocytes 3,410,000, thrombocytes 13,600 and plasma carbon dioxide 51.5 volumes per cent. No icterus was noted, and repeated blood cultures continued to be negative.

On December 29 a biopsy of tibial marrow yielded a differential count that was considered approximately normal but (1) the nucleated cells were scarce and (2) the granules in the neutrophilic myelocytes were pseudobasophilic ("toxic"). It was considered that the clinical course, as well as the blood and the marrow, except the neutropenia, were improving, and continuation of supportive therapy was advised. Additional laboratory studies showed the blood urea nitrogen to be 11 mg, the serum proteins 4.5 Gm (albumin-globulin ratio 1.4) and the blood sugar 65 mg per hundred cubic centimeters. A roentgenologic survey of the skeleton revealed no roentgen evidence of any abnormality in density, texture or contour of the various bony structures.

The febrile course continued, but no new petechiae were noted, although the lesions over the dorsum of the body were becoming confluent. On January 2 another 250 cc of whole blood was given because of decline of the erythrocytes to 2,500,000 and of the hemoglobin to 8.2 Gm. The healing of the splenectomy incision continued to be slow. On January 3 a roentgenogram of the chest revealed some density supra-adjacent to the right hilus, which was interpreted as pneumonitis or pneumonia confined to the apical segment of the lower lobe of the right lung. The hematologic picture revealed only a slow decline, and the child appeared to be showing gradual clinical improvement.

This transient period of clinical improvement did not continue. On January 13 a new crop of petechial hemorrhages was observed over both temporal regions and over the upper part of the trunk. The following day tibial marrow and

6 Doan, C. A., and Wright, C. S. Blood 1 10, 1946

spinal fluid were removed for routine examinations as well as for special studies for *Histoplasma* and other fungi. The spinal fluid was reported as containing 18 mg of protein and 122 mg of sugar per hundred cubic centimeters, with no bacterial growth on aerobic, anaerobic and carbon dioxide cultures. The marrow films could not be interpreted. Many large, degenerated cells with vacuolated cytoplasm and fragmented nuclei were observed. These gave the appearance of "overheated," degenerated cells, conditions due to improper preparation. Insufficient cells were recognizable to make a differential count. "Gaucher's cells" were considered, but none sufficiently characteristic were visualized. Another aspiration of marrow was requested.

On January 16 the erythrocyte count was 3,100,000 and the hemoglobin value was 9.6 Gm, the leukocyte count was 3,800, with neutrophilic myelocytes 29 per cent, lymphocytes 68 per cent and monocytes 3 per cent. No thrombocytes were seen, and 27 nucleated erythrocytes per hundred leukocytes were noted. A transfusion of whole blood, 170 cc, was given, and another 170 cc was given on the following day. Administration of folic acid, a vitamin K analogue (synkayvite® [tetrasodium, 2-methyl-1, 4-naphthohydroquinone, diphosphoric acid ester]), ascorbic acid, a multivitamin preparation, penicillin, desoxycorticosterone acetate and supplemental parenteral protein hydrolysates (amigen®) was continued. Fecal cultures were negative for non-lactose-fermenting organisms, and agglutination tests for typhoid and paratyphoid were negative. New petechiae continued to appear and now covered almost all of the skin surfaces, being most marked on the head and the trunk. The general condition of the patient continued to be unsatisfactory.

On January 20 the tibial marrow revealed very few nucleated red cells and a differential count similar to the differential count of the peripheral blood of the same date. The question of aplastic marrow was considered. Again many large cells with foamy cytoplasm were observed, and the strong possibility of a lipid storage disease was proposed.

On January 25 another 100 cc of whole blood was given by transfusion because of a regression in the blood picture. New petechiae continued to appear, the skin became waxy and pallid, and the appetite poor. At this time the diagnosis of Letterer-Siwe disease was considered most likely. Another roentgenologic skeletal survey was made, which revealed some demineralization as contrasted with the previous examination (four weeks prior to this). The demineralization was considered nonspecific and was best seen in the metaphyseal areas of several of the long bones. Another 250 cc of blood was given by transfusion on January 27 because of a hemoglobin value of 4.9 Gm and an erythrocyte count of 1,650,000. Only slight clinical improvement followed. Smears and cultures of the spinal fluid and tibial marrow were reported negative for *Histoplasma* and other fungi. The petechiae became more confluent, the child became progressively paler and weaker, the respiratory rate increased, and on February 2 the patient died, fifty-four days after the onset of symptoms.

Autopsy—The subject was a thin white girl who weighed 15 pounds (6.8 Kg). Numerous dark and recent petechiae were present over the entire body, most marked over both lower extremities and over both forearms, at which sites they were almost confluent (fig 1). There was slight pitting edema of the vulva and both lower extremities. Petechiae were noted in the conjunctivas, and the gingivae had recent small hemorrhagic ulcerations. The oral mucous membranes were pallid, and petechiae were observed on the hard and soft palates. A granulating surgical incision was present in the left upper quadrant of the abdomen. No peripheral lymphadenopathy or icterus was present.

The serosal surfaces of the thoracic viscera were of a light gray-yellow pallor. The pericardium contained approximately 10 cc of clear, light amber fluid. Fibrinous adhesions were present between the various serosal surfaces in the left upper region of the abdominal cavity. The serosal surfaces of the large bowel



Fig 1—Patient showing generalized hemorrhagic manifestations and a granulating abdominal surgical wound. Note the confluence of lesions on the forearm.

showed numerous diffuse dusky darkened areas, some of which were confluent, grossly suggesting submucosal hemorrhages.

The heart weighed 39 Gm (normal, 44 Gm) and was grossly normal except for a definite pallor of the myocardium. The right lung weighed 77 Gm, the

left, 67 Gm (normal, 64 to 57 Gm) The pleural surfaces were finely lobulated and presented a smooth, very pale yellow-gray appearance All lobes were firm and elastic on palpation, and cut sections again presented rather homogeneous, pale yellow-gray surfaces

The adrenal glands showed a definite pallor The right kidney weighed 27 Gm, the left, 42 Gm (normal, 36 Gm) The cortical surfaces and the cut sections were not unusual except for a firm consistency and a pallid appearance

The liver was grossly enlarged and weighed 400 Gm (normal, 288 Gm) The organ was firm, and cut sections revealed a pale tawny parenchyma

The entire large bowel contained a considerable amount of tarry fecal material The submucosa of the large intestine presented numerous patchy, circumscribed, at times confluent, areas of purple discoloration which grossly suggested submucosal hemorrhage The major finding in the abdominal cavity was the marked enlargement of the periaortic and mesenteric lymph nodes The nodes measured up to 2 cm in diameter and for the most part were matted and of a semirubbery consistency The cut surfaces were pale yellow-brown, and interspersed were numerous small and confluent areas of recent and old hemorrhage

The brain weighed 750 Gm (normal, 925 Gm) Over both cortical surfaces several small punctate areas of old hemorrhage were noted After fixation and coronal sectioning of the brain, the described punctate areas of hemorrhage appeared to be confined to the cortical aspects of the hemispheres, the deeper structures being grossly uninvolved

The marrow of the ribs, long bones and vertebral bodies was light tan and of homogeneous appearance Except for distinct pallor, gross observations of the other structures and viscera revealed nothing remarkable

Microscopic Observations—Microscopic examination of the brain revealed small irregular areas of recent and old hemorrhage in the cortical and subcortical regions These measured up to 2 mm in diameter and were confined to the peripheral portions of the cerebral hemispheres, the deeper structures, the cerebellum and the brain stem remained uninvolved

All the lymph nodes examined presented an almost identical picture The normal structure was effaced by the presence of large numbers of atypical mononuclear cells (fig 2) The nodes were traversed by long stringy bands of collagenous tissue in which a few intermingling fibroblasts could be observed Germinal centers could not be recognized, and the normal lymphoid elements were almost completely replaced by accumulations of loosely arranged atypical mononuclear cells The predominant cells were of irregular outline with acidophilic cytoplasm The nuclei were oval, lobulated, irregular or indented and eccentrically located The intensity of nuclear staining varied, the smaller nuclei staining darkly, whereas the larger nuclei were vesicular and stained lighter The cytoplasm was eosinophilic and finely granular and in many cells was finely vacuolated Multinucleated cells were rare Some of the cells showed phagocytosis of dark brown pigment granules and contained small vacuoles These cells were most prominent in the peripheral sinuses Rare small collections of large cells with pale, eccentrically placed nuclei and foamy cytoplasm were noted in perivascular locations in the central portions of a few of the nodes examined Small areas of recent and old hemorrhage were noted in some of the nodes, and in many there were small foci of necrosis These granulomatous-like areas were conspicuous because of the lack of neutrophilic myelocytic or lymphocytic response at their periphery Sections stained with scarlet red showed that the cytoplasm of some of the large foam cells in the perivascular sites contained small amounts of lipid material Osmic acid did not stain these cells Reticulum stains revealed marked proliferation of reticulum throughout all of the nodes Masson's stain was not

particularly helpful but again demonstrated the prominent stringy bands of connective tissue. Frozen sections examined under crossed Nicol prisms did not show any doubly refractile bodies.

The thymus gland could not be recognized as such because the normal tissue had been almost completely replaced by a loose collagenous network which contained small numbers of fibroblasts. Small areas of recent and old hemorrhage with focal necrosis and scattered macrophages containing pigment granules were noted throughout the parenchyma. A diffuse sprinkling of small cells with darkly staining oval, irregular or elongated nuclei and sparse cytoplasm was seen, most evident near sites of capillary proliferation. Numerous atypical cells were again in abundance, many with pale granular cytoplasm and with most of their nuclei pale staining and lobular or greatly indented. Scattered multinucleated giant

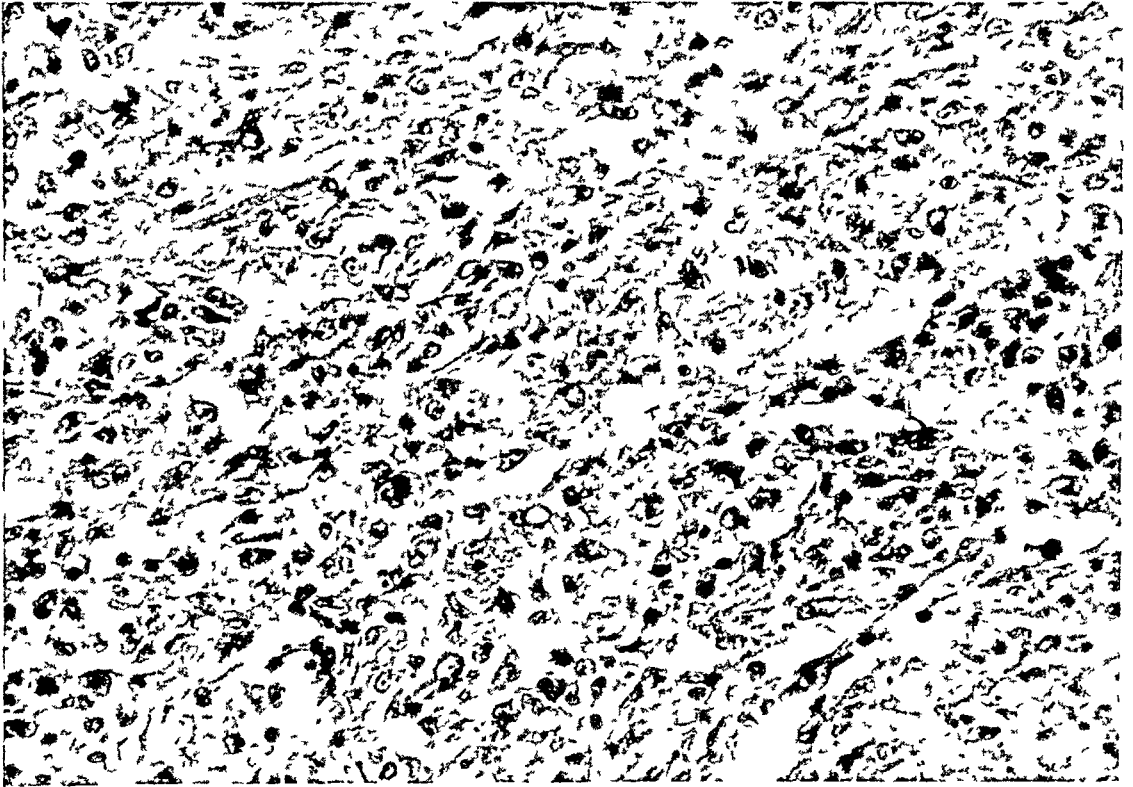


Fig 2—Lymph node showing diffuse involvement. The normal structure has been replaced by atypical mononuclear cells. Hematoxylin and eosin, high power magnification.

cells were noted (fig 3). Only an occasional large cell with foamy cytoplasm was encountered. Frozen sections did not stain with scarlet red or with osmic acid, and tissues examined with polarized light revealed no abnormality.

Several of the periportal areas of the liver were conspicuous because of small aggregates of atypical mononuclear cells. Occasional small collections were noted adjacent to some of the bile ducts, while at other sites, not related to any of the periportal areas, similar small collections were present. The Kupffer cells were prominent, and many were heavily laden with dark brown pigment. The sinusoids were moderately dilated and congested, and in a few areas the sinusoids were very prominent and contained large polyhedral cells which appeared to be liberated and degenerating liver cells.

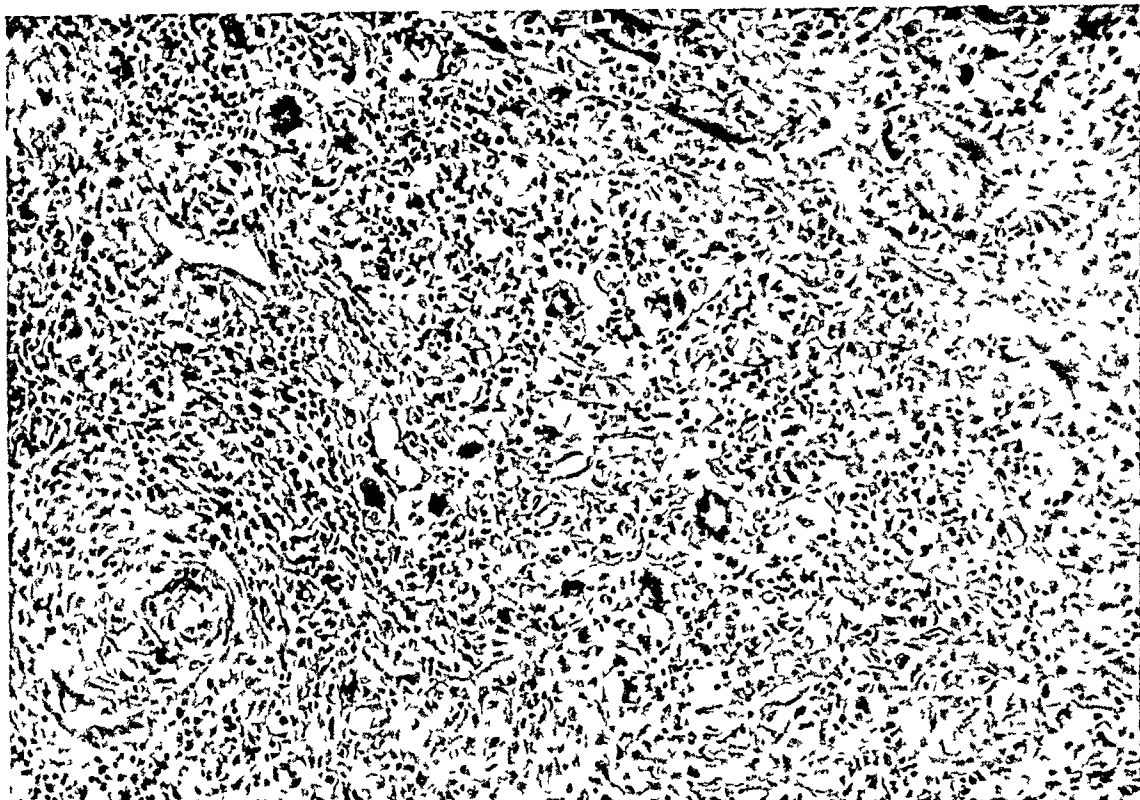


Fig 3—Thymus showing numerous giant cells and collections of atypical mononuclear cells that completely alter the normal pattern Hematoxylin and eosin, low power magnification

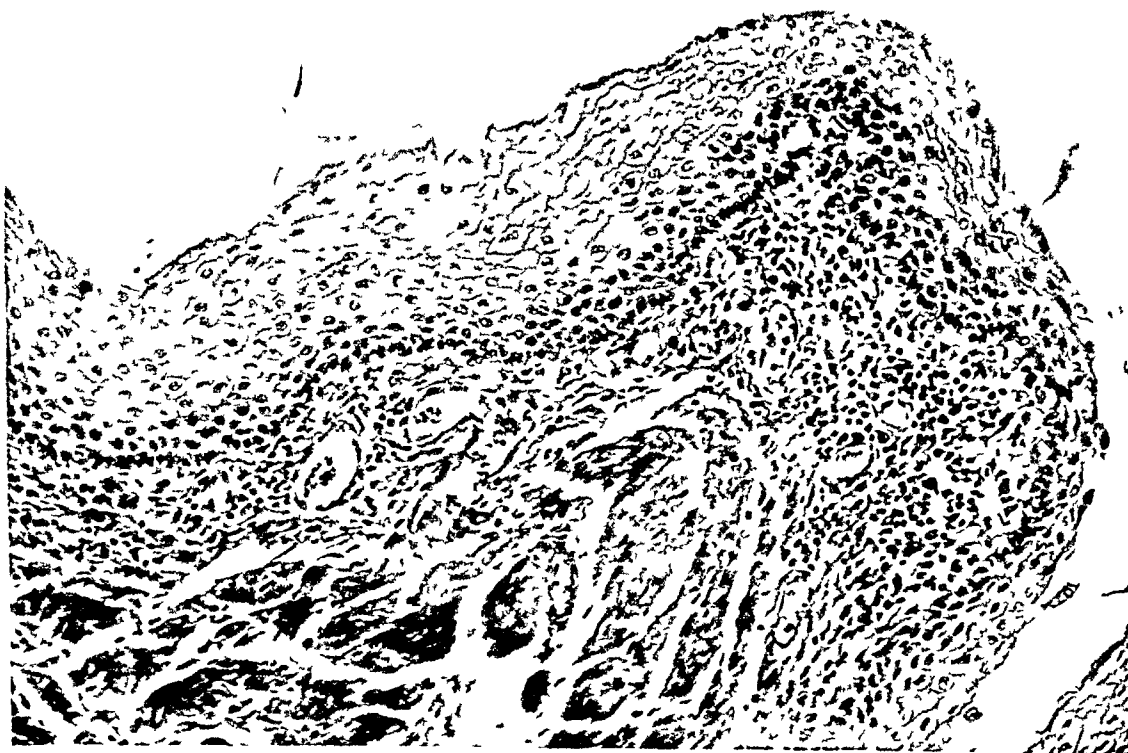


Fig 4—Cutaneous lesion showing collections of mononuclear reticuloendothelial cells in the corium Hematoxylin and eosin, microtessar

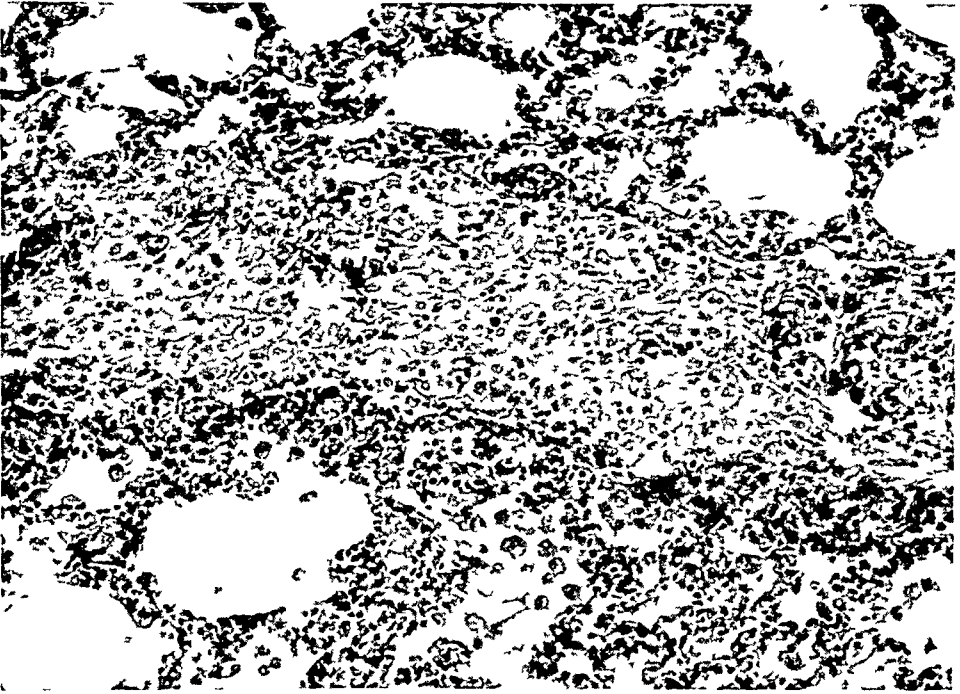


Fig 5—Lung with traversing band of large atypical cells. Pleural surfaces presented a similar appearance. Hematoxylin and eosin, high power magnification.



Fig 6—Marrow with normal elements almost completely replaced by diffuse collections of mononuclear reticuloendothelial cells. Hematoxylin and eosin, high power magnification.

The epithelium of the skin showed patchy areas of thinning with collections of similar atypical mononuclear cells and lymphocytes in abundance in the corium (fig 4) Small areas of recent and old hemorrhage were also noted

The submucosa of the large intestine revealed small areas of hemorrhage and interglandular collections of atypical mononuclear cells The normal lymphoid follicles were almost completely replaced by similar cells and occasional larger macrophages In some areas the overlying mucosa was thinned and ulcerated

The pleura was thickened by loose collections of large, atypical mononuclear cells with pale, granular cytoplasm, and the interlobular septums of the lung were conspicuous and widened by bands and sheets of similar cells (fig 5) The perivascular and peribronchial regions presented focal accumulations of similar cells A few large, pigment-laden macrophages were present in some of the alveolar spaces Fat stains were negative, and examination with Nicol prisms revealed no doubly refractile bodies

Sections from the sternum, vertebral bodies and long bones revealed essentially similar changes There was almost complete destruction of the normal hemopoietic cellular elements by similar atypical mononuclear cells (fig 6) Occasional large macrophages with vacuolated cytoplasm were present Giemsa stains demonstrated the decrease in normal cellular components more prominently than hematoxylin and eosin

COMMENT

The clinical course and the anatomic findings in this case leave little doubt that it represents a disease of the reticuloendothelial system known as Letterer-Siwe disease

Three weeks prior to the onset of the acute phase of the disease the patient had a "throat infection" which rapidly responded to sulfonamide therapy Whether this illness was related to the development of the subsequent disease is not known, and no definite infectious agent can be directly implicated

The patient was an infant, and the onset was acute and characterized by hemorrhagic phenomena, fever, splenomegaly and hepatomegaly There was no familial history The course of the disease was rapid and accompanied by severe anemia These may be regarded as typical clinical findings Only a few small posterior cervical nodes were palpable early in the disease, however, marked mesenteric lymphadenopathy was present at the termination of the illness

Acute leukemia was suspected at the time of admission on the basis of the clinical findings and a peripheral blood smear which was interpreted to show numerous "blast forms" In addition to other immediate therapy a small amount (1 mg) of aminopterin was given However, the original diagnosis was not verified a few hours later when a competent hematologist viewed the peripheral blood and marrow slides and noted that the previously reported "blast forms" were in reality nucleated erythrocytes, which were present in great numbers It is known that aminopterin exerts suppressive effects on the lymphoid and myeloid elements⁷ Because the total amount of this agent given was so small

7 Farber, S Blood 4 160, 1949

and because subsequent therapy probably nullified any obvious effects, it was felt that this drug did not in any way materially alter the clinical course and the gross and microscopic alterations in this case

The cause of the fever remained obscure until the disease was well advanced. Pathologic examination of the removed spleen revealed large histiocytes in some of the dilated sinusoids, but the remainder of the organ was so completely effaced by areas of hemorrhage, infarction and hemopoiesis that the import of these cellular collections was not fully realized at that time

The original skeletal survey showed nothing of importance, and a subsequent one, twenty-five days later, revealed only minor nonspecific demineralizations of the metaphysial areas of several of the long bones

The peripheral blood revealed a rather constant progressive severe anemia with numerous nucleated red cells. The leukocyte count was constantly low, with a relative lymphocytosis, accompanied by marked lowering of the thrombocytic level. The marrow was studied on four occasions because of the lack of a definitive diagnosis. The first two studies were not diagnostic, the third revealed large cells which were interpreted as an artefact, but on the fourth examination these cells were again in evidence and the likelihood of a lipid storage type of disease was strongly suspected

Bacteriologic studies of blood, urine, feces, marrow and spinal fluid, as well as other studies for fungi and histoplasma, gave constantly negative results. Intensive therapy did not alter the progressive course of the disease

The reticuloendothelial apparatus has a wide distribution and serves a diversity of functions. It is primarily composed of three main types of cells—monocytes, histiocytes and the reticuloendothelial cells, proper. The latter may be cells attached to the reticulum of the organs of hemopoiesis or endothelial cells that line the sinusoids of the liver, lymph nodes, marrow and spleen. The main function of this collection of cells is varied but includes phagocytosis, the formation of blood cells, the production of antibodies and also storage. Many enigmas and heterogeneous disease processes have been attributed to various alterations of this system, and many have been grouped under the all-inclusive term "reticuloendotheliosis." These have included some infectious diseases, such as tuberculosis, malaria and typhoid, the so-called lipid storage diseases, such as Gaucher's disease (kerasin), Neimann-Pick disease (phosphatide) and Schuller-Christian disease (cholesterol), xanthomatosis and diabetic lipemia, certain leukemias, for instance monocytic leukemia, neoplastic diseases, such as reticulum cell sarcoma, Hodgkin's disease, and, finally, Letterer-Siwe disease.

The original criteria for Letterer-Siwe disease² (also called non-lipoid reticuloendotheliosis and aleukemic reticulosis) included a pro-

liferation of the cells of the reticuloendothelial system due to a noninfectious cause, thus tending to separate it from the infectious group. Some have adhered to the original premise.⁸ Other writers,⁹ in reviewing their own cases as well as some of those of others that had been previously reported, have expressed the belief that infection plays either a primary or a secondary causative role. Wallgren¹⁰ challenged the belief that the infectious and the noninfectious reticuloendothelioses represent separate entities.

The original criteria² also included the observation that the macrophages were nonlipid storing. In 1940 Glanzman¹¹ reported a case in which lipid material was seen in some of the granulomatous lesions, an observation that led him to believe that the disease was closely related to Schuller-Christian disease. Others¹² also have postulated or suggested that overlapping occurred between the two diseases. Several cases¹³ in which the disease processes represent intermediate, mixed or borderline forms strongly support this view.

Green and Farber¹⁴ made an attempt to correlate Letterer-Siwe disease with other diseases of the reticuloendothelial system, namely, Schuller-Christian disease and eosinophilic granuloma of bone because of histologic similarities noted in various stages of these disease proc-

8 (a) Akiba^{3b} (b) Prodvinec, E, and Terplan, K. *Arch f Kinderh* **93** 40, 1931, cited by Abt and Denenholz.⁴ (c) Siwe.² (d) Foot, N. C., and Olcott, C. T. *Am J Path* **10** 81, 1934. (e) Abt and Denenholz.⁴ (f) Schafer.⁵

9 (a) Letterer.¹ (b) Schultz, A., Wermbter, F., and Puff, H. *Virchows Arch f path Anat* **252** 519, 1924, cited by Schafer.⁵ (c) Krahn, H. *Deutsches Arch f klin Med* **152** 179, 1926, cited by Wallgren.¹⁰ (d) Akiba.^{3b} (e) Sherman, I. *Arch Path* **7** 78, 1929. (f) Guizzetti.^{3c} (g) Prodvinec and Terplan.^{3b} (h) Gittens, R. *Arch Dis Childhood* **8** 367, 1933. (i) Uher, V. *Virchows Arch f path Anat* **289** 504, 1933. (j) Klostermeyer, W. *Beitr z path Anat u z allg Path* **93** 1, 1934, cited by Wallgren.¹⁰ (k) Foot and Olcott.^{8d} (l) Mallory, T. B. *New England J Med* **227** 955, 1942. (m) Jaffe, H. L., and Lichtenstein, L. *Arch Path* **37** 99, 1944.

10 Wallgren, A. *Am J Dis Child* **60** 471, 1940.

11 Glanzman, E. *Ann pædiat* **155** 1, 1940.

12 (a) Glanzman.¹¹ (b) Wallgren.¹⁰ (c) Freud, P., Grossman, L., and Dragutsky, D. *Am J Dis Child* **62** 776, 1941. (d) Gross, P., and Jacob, H. W. *Am J M Sc* **203** 673, 1942.

13 (a) Schultz and others.^{9b} (b) Erber, L. J. *Virchows Arch f path Anat* **282** 621, 1931. (c) Merritt, K. K., and Page, B. H. *Am J Dis Child* **46** 1368, 1933. (d) Grady, H. G., and Stewart, H. L. *Arch Path* **18** 699, 1934. (e) Galeotti Flori, A., and Parenti, G. C. *Riv di clin pediat* **35** 193, 1937, cited by Freund and Ripps.^{13h} (f) Lane, C. W., and Smith, M. G. *Arch Dermat & Syph* **39** 617, 1939. (g) Sweitzer, S. E., Winer, L. H., and Cummings, H. A. *ibid* **40** 192, 1939. (h) Freund, M., and Ripps, M. L. *Am J Dis Child* **61** 759, 1941.

14 Green, W. T., and Farber, S. *J Bone & Joint Surg* **24** 499, 1942.

esses Jaffe and Lichtenstein¹⁵ and others¹⁶ have believed that they represent varying gradations of severity of the same basic process an acute and fatal form, in which the reticuloendothelial cells show little or no lipid deposition (Letterer-Siwe disease), a chronic form, with a protracted clinical course, characterized by typical foam cells containing cholesterol (Schuller-Christian disease), and, finally, a benign form of the same basic process in which recovery is apt to occur (eosinophilic granuloma) It is true that all have some clinical, roentgenographic and morphologic features which suggest that they may represent different phases of the same basic disorder¹⁶

In the literature it is at once apparent that there are numerous opinions as to the proper classification, the cause and the true nature of this unusual proliferation of the reticuloendothelial system One who would take a fixed position on this yet unsettled condition would indeed be intrepid The reader is referred to a recent excellent review by Schafer⁵ for a more complete treatment of this subject

SUMMARY

A case of Letterer-Siwe disease (nonlipid reticuloendotheliosis) is presented It is believed that in this case the disease was initiated by an infectious process that occurred three weeks prior to the onset of the acute symptoms

Similarities noted in roentgenographic, morphologic and some clinical features strongly suggest that Letterer-Siwe disease, Schuller-Christian disease and eosinophilic granuloma represent similar types of a fundamental basic disorder of the reticuloendothelial system

15 Jaffe, H L., and Lichtenstein, L Arch Path **37** 99, 1944

16 (a) Farber, S Am J Path **17** 625, 1941 (b) Mallory⁹¹ (c) Gross and Jacob^{12d} (d) Curtis, A C., and Crowley, E P Arch Dermat & Syph **55** 810, 1947 (e) Dundon, C C., Williams, H A., and Laipply, T C Radiology **47** 433, 1946 (f) Guizetti^{3c}, cited by Gross and Jacob^{12d} (g) Straus, B Am J Med **5** 245, 1948

THE SYNDROMES OF THE CEREBRAL ARTERIES

FAE TICHY, M D

MINNEAPOLIS

THE STUDY of the syndromes resulting from occlusions of the various cerebral arteries is frequently neglected because it does not have immediate clinical application. This disregard also may be attributed to the diversity of opinions and the inadequate consideration given the subject in the standard references. Yet exact information on this subject is critical for cerebral localization and yields a practical framework for experimental and surgical procedures as well as having direct prognostic and therapeutic significance.

A number of methods have been employed in the investigation of the cerebral arteries. Their fields of distribution have been determined by dissection, injection of dyes, and comparative studies of species lower in the phylogenetic series. Correlations of the clinical and pathologic observations have produced descriptions of typical syndromes. However, these are often confusing unless cognizance is given to certain factors responsible for variations in the clinical picture. These include anomalies of the vessels, collateral circulation, cerebral dominance, ability of intact parts to undertake new functions, effect of diaschisis, variations in the site of occlusion of any one vessel, the presence of more than one occlusion, cerebral arteriosclerosis or other pathologic processes, variations in the size of the areas of cortical representation, overlap of the fields of distribution of the various vessels.

In the present work an effort has been made to review and clarify the available literature and include the anatomic and clinical aspects in outline form. The outline form was chosen as being most suitable not only to stress the cardinal features but also to include the minor details which the reader might wish for reference. The sections on the fields of distribution of the various vessels are derived mainly from the excellent work of Beever,¹ that on the anterior cerebral artery from Critchley,² and that on the anterior choroidal artery from Abbie.³

From the Division of Neurology, University of Minnesota Medical School

1 Beever, C E Brain **30** 403, 1907

2 Critchley, M Brain **53** 120, 1930

3 Abbie, A A Brain **56** 233, 1933

THE INTERNAL CAROTID ARTERY

Course—The internal carotid artery arises from the common carotid artery at the level of the upper border of the thyroid cartilage. It passes through the carotid canal in the petrous portion of the temporal bone and through the foramen lacerum to reach the cranial cavity. It continues to the anterior clinoid process, where it pierces the dura mater and attains the base of the brain at the beginning of the fissure of Sylvius. Here it rests on the anterior perforated substance and lies between the optic and the oculomotor nerves.

Branches—1 The ophthalmic artery is the only large branch given off by the internal carotid artery before it reaches the brain. The branch passes through the optic foramen and sends further branches to the ocular muscles, the retina and the lacrimal apparatus.

2 Terminal branches. These include the anterior cerebral artery, the middle cerebral artery, the posterior communicating artery and the anterior choroidal artery.

Clinical Significance—Occlusion of the internal carotid artery may cause no or minimal clinical signs if the circle of Willis is competent (Brock⁴). However, rapid closure of the vessel may result in convulsions and death in some patients. Contralateral hemiplegia and hemianesthesia plus complete aphasia (if the closure is in the major hemisphere) may occur. Involvement of the ophthalmic artery causes ipsilateral blindness⁴. The lodging of a thrombus or an embolus in the internal carotid artery at the origin of the ophthalmic artery results in contralateral hemiplegia with ipsilateral primary optic atrophy (Alpers⁵).

THE ANTERIOR CEREBRAL ARTERY

Course—From its origin, the anterior cerebral artery first courses anteriorly and medially along the anterior perforated substance, then between the olfactory tubercles and the optic nerves to lie at the margin of the mesial and orbital surfaces of the hemisphere. Here it connects with the corresponding vessel of the opposite side via the anterior communicating artery. It then turns up and forward over the genu of the corpus callosum and courses along the epicallous sulcus. It ends by going up obliquely along the parietal lobe to the square or quadrangle lobe or to the parieto-occipital fissure.

Field of Distribution—1 The entire mesial surface of the frontal and parietal lobes to a depth of 2.5 cm.

4 Brock, S. J. *Basis of Clinical Neurology*, Baltimore, William Wood & Company, 1937.

5 Alpers, B. J. *Vascular Diseases of the Brain* in Tice, F. *Practice of Medicine*, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 10.

2 The orbital surface of the frontal lobe from the midline to the external limit of the internal orbital convolution

3 The genu and anterior four fifths of the corpus callosum

4 The septum pellucidum, upper parts of the anterior pillars of the fornix, and the medial parts of the anterior commissure

5 The inferior aspect of the anterior portion of the head of the caudate nucleus, of the anterior part of the two outer segments of the lenticular nucleus, and of the anterior one half of the forelimb of the internal capsule

Branches—1 Basal branches There are three or four branches which pierce the anterior perforated substance to supply the head of the caudate nucleus One of these, the recurrent artery of Heubner, supplies the following structures

(a) Olfactory peduncle

(b) The anterior part of the caudate nucleus

(c) The anterior third of the putamen

(d) Tip of the outer segment of the globus pallidus

(e) The anterior limb of the internal capsule

(f) Rubenstein⁶ included the external capsule and the genu of the internal capsule in the distribution of the recurrent artery of Heubner

2 Anterior communicating artery This has no branches

3 Branches from the convexity

(a) The prefrontal branch is the largest and goes to the medial aspect of the frontal lobe and supplies Brodmann's area 11

(b) The frontopolaris branch supplies the superior part of the superior frontal gyrus (Brodmann's area 10)

(c) The anterior internal frontal branch arises at the level of the genu and supplies the mesial part of area 9

(d) The middle internal frontal branch ends in the upper or posterior end of the superior frontal gyrus (area 8)

(e) The posterior internal frontal branch ends in the uppermost limit of the precentral fissure (area 6)

(f) The paracentral artery ends in the paracentral lobule

(g) Superior parietal branches are also occasionally present These end in the postcentral cortex and superior parietal convolutions

(h) The precuneal branch supplies the mesial part of area 7

⁶ Rubenstein, H S Arch Neurol & Psychiat 52 526, 1944

- (1) Occasionally the anterior cerebral artery ends as the parieto-occipital branch which ends in the parieto-occipital fissure

4 Branches from the concavity consist of a number of short twigs to the genu and body of the corpus callosum. Some of these penetrate and supply the septum pellucidum, part of the anterior commissure and the anterior pillars of the fornix.

Clinical Significance—The syndromes vary according to the sites of the artery at which occlusions occur.

1 Total occlusion of the anterior cerebral artery (including Heubner's artery)

- (a) Contralateral severe hemiplegia due to involvement of the anterior part of the internal capsule and the paracentral lobule. Involvement of the internal capsule causes paralysis of the contralateral half of the tongue and lower part of the face, and the arm, especially of the proximal part of the arm. Damage to the paracentral lobule results in paralysis of the leg, especially in its distal part.
- (b) Mild sensory loss over the paralyzed lower extremity due to involvement of the mesial part of the postcentral gyrus.
- (c) If the hemiplegia is right-sided, left-sided ideomotor apraxia appears. Critchley² expressed the belief that this is due to the involvement of the corpus callosum. If the right anterior cerebral artery is affected, the left-sided apraxia will be masked by the severe motor paralysis of the extremity and the right extremities will be normal in this case, according to Critchley. Nielson⁷ attributed ideokinetic apraxia to lesions of the corpus callosum as well as to lesions of the major supramarginal gyrus (which is supplied by a branch of the middle cerebral artery).
- (d) Mental changes—retardation, confusion, disorientation, and occasionally dementia, witzelsucht and coma. Incontinence of urine and feces may occur. All these findings are attributed to callosal and frontal lobe involvement. The incontinence may be due to involvement of the paracentral lobule or related to the mental changes.
- (e) Aphasia occurs if the lesion is in the major hemisphere. It is of the motor type and usually temporary and mild.

2 Occlusion of the anterior cerebral artery after emergence of the recurrent artery of Heubner and the anterior communicating artery

⁷ Nielson, J. M. *Agnosia, Apraxia, Aphasia*, New York, Paul B. Hoeber, Inc., 1946.

- (a) Hemiplegia with crural predominance due to direct involvement of the leg area of the paracentral lobule. This hemiplegia is often of the flaccid type,⁴ which may indicate that the lesion is limited to area 4 and spares area 6.
- (b) Indefinite sensory impairment in the affected part, especially the leg, due to involvement of the postcentral gyrus.
- (c) Left-sided apraxia regardless of which hemisphere is affected.
- (d) Mental changes—retardation, confusion, occasionally coma.
- (e) Aphasia if the lesion is on the major side. This is usually transient and associated with dysarthria. In a case in Critchley's² series there were also echolalia and palilalia.
- (f) Forced grasping and groping movements may be noted in the hemiplegic upper extremity.

3 Occlusion of the individual branches of the anterior cerebral artery

- (a) Occlusion of Heubner's artery. Critchley² reported a case in which occlusion of Heubner's artery occurred with resultant weakness of the contralateral arm, mild weakness of face, palate and tongue, and aphasia. The lesion was in the left (major) hemisphere. According to Brock,⁴ occlusion of the recurrent artery of Heubner causes paralysis of the contralateral shoulder, the lower part of the face and the tongue and an extrapyramidal type of rigidity or involuntary movement.
- (b) Occlusion of the middle and posterior internal frontal branches. Critchley² reported a case in which these occlusions occurred, and the patient suffered contralateral hemiplegia, with eyes and head deviated to the opposite side.
- (c) Occlusion of the paracentral artery. This results in weakness of the contralateral lower limb, or crural monoplegia. The weakness is greatest in the distal parts of the limb. Occasionally paresis of the contralateral arm and sides of face and tongue may occur.

In conclusion one can say that the outstanding symptoms from occlusion of the anterior cerebral artery include (1) paralysis of the contralateral lower extremity, (2) forced grasping and groping movements of the upper limb, (3) ideokinetic apraxia affecting the left arm, whether the occlusion be right or left sided, (4) mental changes—deterioration, emotional lability. Thrombosis of the vein of Rolando closely resembles occlusion of the paracentral artery.²

Bilateral lesions of the anterior cerebral artery cause weakness of the legs and almost no sensory change in reported cases. An aneurysm of the anterior cerebral artery near its origin may cause unilateral anosmia, progressive dimness of vision and primary optic atrophy. Aneurysms on the anterior communicating artery may cause bitemporal hemianopsia.

Dandy⁸ expressed the belief that the center of consciousness lay in the left cerebral hemisphere along the mesial aspect of the hemisphere near the anterior part of the corpus callosum. According to him, if the left anterior cerebral artery was injured, the patient could never regain consciousness. Poppen⁹ reported a series of 10 cases in which ligation of the left anterior cerebral artery was done, with success in 8 cases. Bilateral ligation was done successfully in 2 of these. He felt that if the blood pressure were kept within normal limits during the surgical procedure, ligation could be done without untoward changes in the state of consciousness and the collateral circulation would be adequate if anemia were not produced.

THE MIDDLE CEREBRAL ARTERY

Course—The middle cerebral or sylvian artery, the largest terminal branch of the internal carotid artery, courses from its origin across the anterior perforated substance to enter the depth of the fissure of Sylvius and then curves outward to the external surface of the hemisphere. Before reaching the surface it gives off its main collateral branches, which come individually to the surface along the fissure.

Field of Distribution—1 Basal branches

- (a) The superior half of the anterior and posterior divisions of the internal capsule
- (b) The superior half of the head of the caudate nucleus, and the horizontal part of the caudate nucleus
- (c) The external and middle segments of the nucleus lenticularis
- (d) The lateral parts of the anterior commissure

2 Cortical branches

- (a) The superior, middle and inferior temporal gyri
- (b) The angular gyrus
- (c) The supramarginal gyrus
- (d) The superior parietal gyrus

⁸ Dandy, W. E., *The Brain*, in Lewis, D. *The Practice of Surgery* Hagerstown, Md., W. F. Prior Company, Inc., 1932, vol. 12, p. 51.

⁹ Poppen, J. L. *Arch Neurol & Psychiat* **41** 495, 1939.

(e) The inferior three fourths of the precentral and postcentral gyri

(f) The internal orbital gyrus

Branches—1 Perforating branches These are given off at right angles from the trunk at the anterior perforated space They penetrate perpendicularly through it They are small and numerous Foix and Levy¹⁰ distinguished three groups

(a) The putaminocapsular, supplying the putamen, the internal capsule and the caudate nucleus

(b) A few external pallidal branches to the lateral part of the globus pallidus

(c) Inconstant pallido-optic branches to the globus pallidus and the thalamus

2 Cortical branches

(a) The anterior temporal artery gives branches to the rostral third of the temporal gyri and the insula

(b) The orbital sulcal branches supply the lateral part of the orbital surface of the frontal lobes and the external surface of the inferior frontal convolution except for the opercular area

(c) The artery of the prerolandic fissure supplies the foot and anterior lip of the precentral gyrus, the foot of the middle frontal convolution and the opercular part of the inferior frontal convolution

(d) The artery of the rolandic fissure supplies the posterior border of the precentral gyrus and the most anterior border of the postcentral gyrus

(e) The anterior parietal artery supplies the posterior border of the postcentral gyrus and the anterior portion of the other parietal convolutions

(f) The posterior parietal artery supplies the supramarginal gyrus and posterior part of the parietal lobe

(g) The artery of the angular gyrus is a continuation of the middle cerebral artery and supplies the angular gyrus

(h) The posterior temporal artery irrigates the posterior two thirds of the superior temporal and the posterior half of the middle temporal convolutions

It is important to note that the branches of the middle cerebral artery are predominantly arteries of fissures and irrigate the margins of the two adjacent convolutions

10 Foix C, and Levy, M Rev neurol 34 (pt 2) 1, 1927

Syndromes of the Middle Cerebral Artery—1 Complete occlusion of the middle cerebral artery causes contralateral hemiplegia, hemianesthesia and hemianopsia, and global aphasia if the lesion involves the major hemisphere. It is usually fatal.

2 Occlusion of the deep branches of the middle cerebral artery results in destruction of most of the putamen, all of the superior part of the internal capsule and the outer segment of the globus pallidus. The clinical findings vary from a moderate degree of hemiplegia of equal severity in both extremities to severe hemiplegia, usually with more marked involvement of the lower extremity. Hemianopsia or sensory defect does not usually arise. Aphasia can result if the lesion is on the major side. Partial involvement of the perforating arteries constantly produces hemiplegia due to involvement of the internal capsule. Hemichorea also occurs at times, possibly due to involvement of the corpus striatum.

3 Occlusion of the prerolandic artery causes contralateral weakness of the lower part of the face and of the tongue. It occasionally produces slight weakness of the hand (Grinker¹¹). If the major hemisphere is involved, there will be motor aphasia.

4 Occlusion of the rolandic artery causes contralateral hemiplegia of a variable degree.

5 Occlusion of the anterior parietal artery causes contralateral defects in gnostic sensation with slight weakness of the upper extremity. Lesions of the major parietal lobe may also cause amnesic aphasia.⁷

6 Occlusion of the posterior parietal and angular artery causes hemianopsia and mild sensory defects. If on the major side, it also causes aphasia of the type described under lesions of the angular artery. Involvement of the posterior parietal artery causes damage of the supra-marginal gyrus with consequent ideomotor apraxia.⁷

7 Occlusion of the angular artery on the major side causes visual verbal agnosia with agraphia. Gerstmann's syndrome (acalculia, confusion of laterality, "finger agnosia" and agraphia) occurs with lesions at the border of the major angular gyrus on the occipital lobe.⁷

8 Occlusion of both the posterior temporal and the angular arteries causes hemianopsia plus aphasia of one or a combination of the types named under the individual arteries.

9 Occlusion of the posterior temporal artery causes hemianopsia, due to softening of the visual radiation, and aphasia if the lesion is in the major hemisphere. If Wernicke's area is affected, there is acoustic

¹¹ Grinker, R. R. *Neurology*, ed 2, Springfield, Ill., Charles C Thomas, Publisher, 1937.

verbal aphasia⁷ Wernicke's aphasia occurs if the superior temporal convolution is involved⁷

THE ANTERIOR CHOROIDAL ARTERY

Course—The anterior choroidal artery arises from the internal carotid artery between the origins of the posterior communicating and middle cerebral arteries. It then crosses the optic tract and runs posteriorly along the medial border of the tract against the cerebral peduncle. At the lateral geniculate body it divides into many branches, most of which recross the optic tract to enter the inferior horn of the lateral ventricle and reach the choroid plexus. The two terminal branches run posteriorly over the lateral geniculate body to join the posterior cerebral and posterior choroidal arteries.

Field of Distribution—1 The optic tract

2 The anterior third of the pes pedunculi

3 The posterior two thirds to four fifths of the inferior half of the posterior limb of the internal capsule

4 The optic radiation

5 The tail of the caudate nucleus

6 The inner segment of the lenticular nucleus

7 The choroid plexus in the descending and posterior cornua

8 The amygdaloid nucleus

9 The outer part of the anterior commissure

10 The uncinate gyrus

Abbie³ included the substantia nigra, the nucleus ruber, the subthalamic body, the ventral lateral nucleus of the thalamus, the lateral and anterior parts of the lateral geniculate body, the stria terminalis and most of the globus pallidus.

*Branches*¹²—1 Nearest the origin of the vessel, branches arise to supply the tail of the caudate nucleus and the outer border of the anterior commissure.

2 Some vessels reach the uncus gyri hippocampi and supply the posteromedial part of the underlying amygdaloid nucleus. These anastomose with branches of the middle and posterior cerebral arteries.

3 A number of vessels go to the anterior inferior part of the hippocampus and the dentate gyrus. These anastomose with branches of the posterior cerebral artery.

4 More posteriorly a series of branches penetrate the optic tract and then pass dorsally into the base of the brain to reach

(a) The inferior half of the posterior limb of the internal capsule

12 Steegmann, A. T., and Roberts, D. J. J. A. M. A. 104: 1695, 1935

- (b) The internal segment of the lenticular nucleus
- (c) The beginning of the optic and acoustic radiation

5 Bianches reach the cerebral peduncle, where they anastomose with branches from the posterior communicating and posterior cerebral arteries. They supply the middle third of the crus cerebri, the upper part of the substantia nigra and nucleus ruber, the subthalamic body and often the most superficial part of the ventral lateral nucleus of the thalamus.

6 From the main trunk or from one of the terminal divisions arises a constant vessel which enters the stria terminalis and the tail of the caudate nucleus. It then runs posteriorly and dorsally in these to the level of the junction of the body and the temporal horn of the lateral ventricle.

7 Branches to the lateral and anterior aspects of the lateral geniculate body. The medial and posterior aspects of the lateral geniculate body are supplied by the posterior cerebral artery. The macular area in the lateral geniculate body is supplied by both of these vessels.¹³

Clinical Significance—The symptoms resulting from occlusion of the anterior choroidal artery are as follows:

1 Hemiplegia in all cases. In a case reported by Kolisko¹⁴ there was no involvement of the anterior half of the posterior limb of the internal capsule and the hemiplegia probably was on the basis of involvement of the crus.

2 Hemianesthesia. This occurs constantly but is usually incomplete. It involves all forms of sensation.

3 Hemianopsia. This is recorded by Schiff-Wertheimer¹⁵ and Ley¹⁶ but not by Kolisko. In a case of Mackenzie's (reported by Abbie³) in which both anterior choroidal arteries were involved, the hemianopsia was a bilateral homonymous superior quadrantic defect with macular fields spared, indicating involvement of the lateral parts of the lateral geniculate bodies.

At autopsy in patients with occlusion of the anterior choroidal artery there is found degeneration in the posterior limb of the internal capsule, the greater part of the globus pallidus and the cerebral peduncle. Degeneration has also been observed in the lateral aspect of the lateral geniculate.³ A few authors have reported involvement also of the head

13 Abbie, A. A. *Anat.* **67** 491, 1933.

14 Kolisko, A. *Ueber die Beziehung der Arteria choroidea anterior zum hinteren Schenkel der inneren Kapsel des Gehirnes*, Vienna, A. Holder, 1891.

15 Schiff-Wertheimer, S. *Les syndromes hemianopsiques dans le ramollissement cerebral*, Thesis, Paris, no. 584, 1926.

16 Ley, J. *J. de neurol. et de psychiat.* **32** 785 and 895, 1932.

of the caudate nucleus, the anterior commissure and the amygdaloid nucleus

THE POSTERIOR COMMUNICATING ARTERY

Course and Distribution —The posterior communicating artery arises from the internal carotid artery just before it divides into the anterior and the middle cerebral artery. It passes posteriorly to join the posterior cerebral artery. It sends branches to the following structures

- 1 The optic chiasm and the tuber cinereum
- 2 The subthalamic region
- 3 The pes pedunculi—anterior third
- 4 The anterior fifth or third of the posterior division of the internal capsule
- 5 The external and internal nuclei of the thalamus

Clinical Significance —The clinical conditions resulting from occlusion of the posterior communicating artery are variable and indefinite. Brock⁴ reported contralateral mimetic facial paralysis.

THE POSTERIOR CEREBRAL ARTERY

Course —The posterior cerebral arteries arise from the bifurcation of the basilar artery. Each runs outward and posteriorly around the cerebral peduncle. After receiving the posterior communicating branch from the internal carotid artery, it passes on to the inferior surface of the occipital lobe and divides into terminal branches.

Field of Distribution —1 Basal branches

- (a) Mamillary bodies
 - (b) The posterior two thirds of the pes pedunculi
 - (c) The nucleus ruber
 - (d) The thalamus (The posterior half, the anterior nucleus, the anterior superior part of the external nucleus, the posterior half of the external nucleus, and the posterior third of the internal nucleus)
 - (e) The choroid plexus in the lateral ventricles
 - (f) The medial geniculate body and the medial and posterior aspects of the lateral geniculate body
 - (g) The body and posterior parts of the fornices
 - (h) The inferior part of the descending columns of the fornices
 - (i) Alpers⁵ includes the posterior limb of the internal capsule in the field of supply of the posterior cerebral artery
- 2 Cortical branches
- (a) The inferior half of the inferior temporal gyrus

- (b) The medial surface of the fusiform gyrus, the lingual gyrus, the cuneus, and the quadrate gyrus

Branches of the Posterior Cerebral Arteries—1 The anterior temporal branch is directed forward over the inferior surface of the temporal lobe, where it supplies the anterior part of the fusiform and the hippocampal gyrus

2 The posterior temporal branch is distributed to the rest of the inferior surface of the temporal lobe

3 The large posterior occipital branch runs buried in the calcarine fissure and supplies the posteromedial and inferior portions of the occipital lobe, especially the lingual lobule and the cuneus

4 A series of collaterals to the mesencephalon and the basal ganglions. These also supply almost all of the thalamic and peduncular regions, including the lateral half of the cerebral peduncle, the subthalamic region, the red nucleus, the corpus luyssi, and the substantia nigra, the posterior inferior half of the thalamus, the superior cerebellar peduncles, the retrolenticular capsule, and the geniculate bodies

Clinical Significance—The posterior cerebral artery is rarely obliterated at its origin. Symptoms from such an occlusion are rare, since anastomoses via the posterior communicating artery may at times be very large, and peripheral branches of the posterior cerebral artery anastomose freely with neighboring arteries. If occlusion occurs, the patient usually gets crossed hemianopsia, if it is sudden, Brock⁴ reported, temporary bilateral blindness may occur. If the posterior cerebral artery of the major side is occluded, the patient may also show the Charcot-Wilbrand syndrome (visual agnosia and loss of ability to revisualize images⁷). Crossed sensory and motor defects may occur from involvement of the thalamus, the posterior limb of the internal capsule and the cerebral peduncle. According to Foix and Masson,¹⁷ complete occlusion of the posterior cerebral artery on the major side will cause (1) hemianopsia, (2) pure alexia and (3) sensory-motor disturbances—hemiparesis and a thalamic syndrome.

If the lesion is in the other hemisphere, thalamic pain is predominant and alexia is absent.

Partial involvement of the posterior cerebral artery causes alexia, hemianopsia and cortical blindness. Foix termed these partial syndromes "partial anterior pedunculo-thalamo-subthalamic syndromes." He divided them into the following types:

1 Cerebellar peduncular type. This type most commonly shows a homolateral cerebellopyramidal syndrome or a predominantly cerebellar

17 Foix, C, and Masson, A. *Presse med* 31 361, 1923

syndrome associated with involvement of the medial longitudinal fasciculus

2 The thalamic syndrome—monoplegic form This type shows thalamic symptoms, with or without monoplegia, and cerebellar symptoms It is due to occlusion of the branches going to the thalamus and consists of hemiparesis, occasionally complete hemianesthesia, hemichorea and central pain Hemianopsia may also occur

Summary of the Findings Most Common in Patients with Occlusions of the Cerebral Vessels

Vessel	Contralateral Motor Signs	Contralateral Sensory Signs	Aphasia*	Visual Field Defects	Mental Changes	Apraxia
Internal carotid artery	Hemiplegia	Hemianesthesia	Complete	Homonymous hemianopsia, ipsilateral blindness or primary optic atrophy (ophthalmic artery)		
Anterior cerebral artery	Hemiplegia, particularly of proximal part of arm and distal part of leg (leg most affected)	Hypesthesia of leg	Mild, transient motor		Intellectual loss, emotional instability	Left ideokinetic apraxia
Heubner's artery	Hemiplegia (mild VII and XII, and proximal part of arm), extra pyramidal rigidity and involuntary movements		Mild motor			
Paracentral artery	Distal part of leg, occasionally arm, VII and XII					
Middle cerebral artery	Severe hemiplegia (arm most severe), dysarthria	Hemianesthesia	Global	Hemianopsia, if partial, of lower quadrant type		Ideokinetic apraxia
Anterior choroidal artery	Hemiplegia (arm and leg equally affected)	Hemianesthesia, usually incomplete, partial thalamic sensory change, especially in arm		Hemianopsia or upper quadrant defect		
Posterior cerebral artery	Hemiparesis, hemiataxia, choreoathetosis, occasionally Weber's syndrome	Hemianesthesia, thalamic pain	Aphasia, alexia most common	Hemianopsia, complete or partial, lower quadrant defect		

* Aphasia is present only if the lesion is on the major side

3 The syndrome of the suboptic region There are two types of suboptic involvement The first shows Weber's syndrome with pyramidal hemiplegia replaced by cerebellar hemiplegia The lesion is in the inferior part of the red nucleus and involves the medial longitudinal fasciculus The second shows a thalamocerebellar syndrome with occasional hemianopsia It is due to a lesion of the red nucleus extending up into the thalamus

Alpers⁵ included a syndrome of hemiplegia, hemianesthesia and hemianopsia (similar to that described as due to occlusion of the anterior choroidal artery) and attributed it to the branch of the posterior cerebral artery which supplies the posterior limb of the internal capsule Nielson⁷

contended that a lesion of the major occipital lobe causes visual autotopagnosia and optical disorientation in space

Numerous similarities of the various syndromes may be noted in the foregoing summary. However, definite differential points exist and should suffice so that localization may be made with relative ease if adequate study of the patient is made. It is noteworthy that a small lesion produced by occlusion of the anterior choroidal artery may produce clinical symptoms so similar to those of an extensive lesion due to a closure of the middle cerebral artery. Speculation about the size of the lesion as judged by the patient's general state (changes in sensorium) may also aid in differentiation.

The overlap in the fields of distribution of the cerebral vessels is fortunate for the patient but confusing for the diagnostician. For example, the internal capsule receives supplies from four vessels (Beever¹). The inferior half of its anterior limb is supplied by the anterior cerebral artery, the superior half, by the middle cerebral artery. The anterior part of the genu is supplied by the anterior cerebral artery, the posterior half, by the posterior communicating artery. The superior half of the posterior limb is supplied by the middle cerebral artery, the inferior half is supplied by the posterior communicating artery (in the anterior one third) and the anterior choroidal artery (in the posterior two thirds).

SUMMARY

A review of the syndromes of the cerebral vessels is presented in an effort to clarify and organize the available material and thus to enhance the practical application.

News and Notes

Appointment—Russell S Fisher, resident fellow in legal medicine in Harvard Medical School, Boston, has been appointed assistant professor of pathology in the Western Reserve University School of Medicine, Cleveland

Congress on Cancer—The Fifth International Congress for Scientific Research and the Social Fight Against Cancer is to be held at the Sorbonne in Paris, France, July 17 to 22, 1950 A Lacasagne is president of the congress Correspondence can be directed to Prof V LeLorier, Secretary-General of the Congress, 6 Avenue Marceau, Paris 8, France

Research on the Betatron—The United States Public Health Service has awarded \$15,000 to the University of Illinois College of Medicine in support of research studies involving the 22,000,000 volt betatron The grant will be used specifically for the study of the effects of the betatron x-ray beam on bone and cartilage, under the supervision of Roger A Harvey, of the department of radiology, and Granville A Bennett, of the department of pathology

Medicolegal Laboratory—A medicolegal laboratory, operated under a cooperative arrangement of Houston County, the University of Texas and the M D Anderson Foundation, was established recently in Houston, Texas, in the Jefferson Davis Hospital The laboratory is headed by W W Coulter Sr, chief pathologist at the hospital, with Charles A Dwyer Jr, county physician, as assistant The laboratory, which will be used in teaching medicolegal pathology in the University of Texas Postgraduate School of Medicine, is under the general direction of William O Russell, head of the department of pathology of the University of Texas Medical Branch, Galveston

The American Society of Clinical Pathologists will hold its twenty-eighth annual meeting in Chicago at the Drake Hotel on October 12, 13, 14 and 15

The Academy of Forensic Sciences (American Medico-Legal Congress) will hold its second meeting in Lincoln Hall, Northwestern University School of Law, Chicago, on Jan 26 27 and 28, 1950 The meeting will be devoted to a discussion of problems of forensic science and a formal organizational program Address A W Freireich, 180 Hempstead Avenue, Malverne, N Y, or Ralph F Turner, Acting Secretary, Department of Police Administration, Michigan State College, East Lansing Mich

Books Received

HISTOPATHOLOGY OF IRRADIATION FROM EXTERNAL AND INTERNAL SOURCES
Edited by William Bloom, M D, professor of anatomy, Department of Anatomy
and Institute of Radiobiology and Biophysics, University of Chicago National
Nuclear Energy Series, Manhattan Project Technical Section Pp 808, illustrated
Price, \$8 New York, Toronto, London McGraw-Hill Book Company, 1948

This is the first volume to be published of approximately sixty monographs entitled the National Nuclear Energy Series, which will record the research done and the technical methods developed during the war by various groups working under the Manhattan Project or, more recently, the Atomic Energy Commission. This volume is one of a series of monographs called the Plutonium Project Record and is a report of a part of the health work carried on mostly by the Metallurgical Laboratory at Chicago during the three year period from 1942 to 1945.

Each chapter is written by an individual author and is the description of the histologic changes observed in a single organ or an organ system following external or internal application of radiation. This organization allows for ready comparing of lesions in a single organ under different kinds and doses of radiation but makes comparing of different organs under such circumstances difficult. Since this work was done under a single director, the criteria of damage were uniform and quite accurate comparisons can be made among the biologic effects of different types of radiation, i e, roentgen, slow neutron, fast neutron, and alpha, beta and gamma radiation. The abundant amount of data on histologic changes resulting from internal irradiation should be of considerable value to those interested in using radioactive isotopes. The absence of data concerning the metabolism of the radioactive elements makes it impossible to determine the amount of internal radiation delivered to an organ, however, as such information becomes available in the literature the value of this work should be greatly enhanced. Dr Bloom modestly states in the introduction that there is little in this book that can be considered new. However, the descriptions of bone lesions following the administration of bone-seeking radioactive isotopes and the emphasis on the radiosensitivity of the erythroblast seem to the reviewer to be important contributions. Also, some controversial points seem to have been settled, e g, the radiosensitivity of the spermatogonia.

The book, by and large, reports objective findings, and there is little speculation concerning the mechanism of the biologic effects of ionizing radiation. For this reason the casual reader may find it tedious, the introduction and the summaries of each chapter, however, are good and should be interesting and informative to such a reader. The worker interested in specific problems will find it of considerable aid as a reference. The book is printed on slick paper and is copiously illustrated with photomicrographs and a few camera lucida colored drawings. Some of the photomicrographs are not very clear, others are quite good. Errors are gratifyingly few. The bibliography is large and well selected.

When one considers that after the war many of the scientists working for the Manhattan Project were anxious to return to their peacetime pursuits and that the recording of the wartime work necessarily was done quickly and under pressure if it was to be done at all, most of the criticisms that might be leveled at the book are dispelled.

BRONCHIAL CARCINOMA

A Practical Method of Early Diagnosis

K R CROSS, M D

T E CORCORAN, M D

T J COOPER, M D

AND

S N LANDIS, M D

DES MOINES, IOWA

IN THIS PAPER we outline and illustrate with cases our experience in diagnosing bronchogenic carcinoma by examination of bronchial aspirations and washings. This material can be embedded, sectioned and stained. As demonstrated in many illustrations dealing with "the cytologic diagnosis of cancer," and as stated by some, cell groups or islands are actually obtained. These, in our experience, amount to minute biopsy specimens and show less distortion in sections than is produced when smears are prepared. The same criteria of cancer, namely, anaplasia and loss of polarity, can be applied to these minute specimens as to larger ones obtained with the knife. The information contained in the published material dealing with the morphologic aspects of these cancers in smears can also be applied. This method of examination does not require a prohibitive amount of time.

Carcinoma of the lung is of great importance. In our experience, it has been the most frequent cancer encountered exclusive of skin cancer. The treatment is surgical removal, the technic of which has been improved remarkably in the recent past. The feature of successful management which now demands most attention is early diagnosis.

The great importance in suspected cases of cancer of examining exfoliated material from the bronchial tree, whether aspirated through the bronchoscope or collected as sputum, and whether examined in smears or in prepared sections, as emphasized by Hunter and Richardson,¹ has been amply demonstrated. By this method, neoplasms can often be diagnosed before other positive evidence can be elicited.

From the Department of Pathology, Veterans Administration Hospital

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¹ Hunter, W C, and Richardson, H L. Surg, Gynec & Obst 85 275, 1947

Lesions inaccessible to the bronchoscope can also be proved to exist when biopsy is impossible or the specimen inadequate, as pointed out by Gibbon, Clerf, Herbut and DeTuerk,² by Woolner and McDonald³ and as illustrated in all 5 of the cases to be reported here

This method so enhances the likelihood of the early diagnosis which contributes to successful treatment that its use can no longer be restricted to large centers and clinics. The general pathologists in the smaller hospitals and clinics, where most of these patients are first seen, can no longer ignore it.

Cancer of the lung has been diagnosed by examination of fluid from the respiratory tract for many years. The first to report such a case was Hampeln,⁴ in 1887, who examined unstained smears of fresh sputum. In 1918 he⁵ published a second paper concerning 25 cases of pulmonary cancer, in 13 of which carcinoma cells were found in the sputum. He stressed the diagnostic importance of isolated neoplastic cells. Betschardt,⁶ using the technic of blocking and sectioning, studied fragments in sputum and reported a case of bronchogenic carcinoma diagnosed by this method in 1895. With this method other investigators (Sauerbruch,^{7a} Fishberg,^{7b} Weller,^{7c} Sweany^{7d} and Edwards^{7e}) also have made positive diagnoses.

Stockard and Papanicolaou⁸ started a study of the morphologic and cyclic variations of exfoliated cells obtained from the vaginas of guinea pigs. Papanicolaou⁹ reported in 1928 that exfoliated cancer cells had been observed in human vaginal secretions. Little cognizance was taken of this finding until 1943, when Papanicolaou and Traut¹⁰ published their monograph "The Diagnosis of Uterine Cancer by the Vaginal Smear."

Dudgeon and Wrigley¹¹ reported in 1935 that they had applied the wet film technic to the examination of sputum for the early diag-

2 Gibbon, J. H., Jr., Clerf, L. H., Herbut, P. A., and DeTuerk, J. J. *J Thoracic Surg* **17** 419, 1948.

3 Woolner, L. B., and McDonald, J. R. *Surg, Gynec & Obst* **88** 273, 1949.

4 Hampeln, P. *St Petersburg Med Wchnschr* **4** 137, 1887, cited by Woolner and McDonald³.

5 Hampeln, P. *Mitt a d Grenzgeb d Med u Chir* **31** 672, 1918-1919.

6 Betschardt, E. *Virchows Arch f path Anat* **142** 86, 1895.

7 (a) Sauerbruch, E. F. *J A M A* **51** 808, 1908. (b) Fishberg, M. *Arch Int Med* **37** 745, 1926. (c) Weller, C. V. *Arch Path* **7** 478, 1929. (d) Sweany, H. C. *Ann Otol, Rhin & Laryng* **43** 561, 1934. (e) Edwards, A. T. *J Thoracic Surg* **4** 107, 1934.

8 Stockard, C. R., and Papanicolaou, G. N. *Am J Anat* **22** 225, 1917.

9 Papanicolaou, G. N. *Proc Third Race Betterment*, 1928, p. 528.

10 Papanicolaou, G. N., and Traut. *The Diagnosis of Uterine Cancer by the Vaginal Smear*, London, Commonwealth Fund, 1943, pp. 47.

11 Dudgeon, L. S., and Wrigley, C. H. *J Laryng & Otol* **50** 752, 1935.

nosis of bronchogenic carcinoma Their method, devised by Dudgeon and Patrick¹² in 1927, consisted in making films of the suspected fluid, fixing these while wet in Schaudin's fluid and staining with hematoxylin and eosin That method of diagnosis has gained in popularity and is now being used by numerous investigators, some of whom have varied the fixative or the stains The examination of exfoliated cells has now been applied to the diagnosis of cancer of the lungs, uterus, kidneys, bladder, prostate and stomach

The reports of the results obtained by using the wet film technic of Dudgeon and Patrick¹² or Papanicolaou,¹³ with or without minor variations, reveal that the diagnosis can be made in nearly 75 per cent of proved cases of bronchial carcinoma Only reports giving sufficient data as to the total number of patients with carcinoma who were examined and the results of those examinations are included here

Dudgeon and Wrigley¹¹ found cancer cells in the sputum of 26 of 38 patients in whom the presence of bronchogenic carcinoma was proved or probable Gower¹⁴ found cancer cells in the sputum of 36 of 65 patients with carcinoma of the lung Papanicolaou¹⁵ reported a positive diagnosis on the basis of examinations of sputum in 22 of 33 cases of proved or probable cancer of the lungs Wandall,¹⁶ examining sputum, found cancer cells in 84 of 100 cases of proved bronchial carcinoma Farber and others,¹⁷ examining both sputum and bronchial secretions, demonstrated cancer cells in 57 of 71 proved cases of bronchogenic carcinoma They found a close correlation of the results obtained when using both sputum and aspirated bronchial secretions McKay, Ware, Atwood and Harken¹⁸ found neoplastic cells in aspirated bronchial material in 40 of 54 cases of proved bronchogenic carcinoma Herbut and Clerf¹⁹ found neoplastic cells in aspirated bronchial material in 47 of 57 cases of proved carcinoma of the lung In 25 of the last 27 cases their results were positive

By profiting from the efforts of the earlier workers and with increasing experience and confidence, the authors of the more recent reports were able to reveal a greater degree of accuracy than was first thought possible with these methods

It seems unnecessary to repeat descriptions of the specific cytologic features of neoplasms of the bronchial system These have been pre-

12 Dudgeon, L S, and Patrick, C V Brit J Surg **15** 250, 1927

13 Papanicolaou, G N Science **95** 438, 1942

14 Gower, F J S Brit J Surg **30** 193, 1943

15 Papanicolaou, G N J A M A **131** 372, 1946

16 Wandall, H H Acta chir Scandinav (supp 93) **91** 1, 1944

17 Farber, S M, and others Dis of Chest **14** 633, 1948

18 McKay, D G, Ware, P F, Atwood, D A, and Harken, D C Cancer **1** 208, 1948

19 Herbut, P A, and Clerf, L H M Clin North America **30** 1384, 1946

sented, first, in standard textbooks of pathology dealing with the microscopic appearance of these neoplasms, and second, in many excellent reviews of the cytologic diagnosis in which small aggregates and single cells are considered ²⁰ This is well covered by Woolner and McDonald,³ and by Diggs,²¹ whose illustrations are excellent During the period covered in this report we have confined our diagnoses simply to suspicions of and the presence or the absence of cancer Wandall¹⁶ and others have gone further in typing these neoplasms on the basis of examinations of sputum or of bronchial secretions We believe this is possible in the majority of cases, and the matter is under further study at this time

It may be noted that in the examination of this material loss of polarity in cell groups and anaplasia of epithelial cells are the most important features to evaluate Inasmuch as one may be dealing with a squamous cell carcinoma, an adenocarcinoma or a small, undifferentiated cell carcinoma, these features must be evaluated in each case Variation from cell to cell in nuclear size, nuclear contour and density of the nuclear membrane—the last varying not only from cell to cell but from one zone to another in the same nucleus—and variation in the arrangement and density of the chromatin and in the size and position of the nucleoli thus become of the greatest importance This variation from cell to cell becomes the key in this diagnosis and is much easier to evaluate when relatively undistorted cell groups are examined and adjacent cells can be compared Wandall¹⁶ in 1944 stressed these variations and concluded that rarely could the diagnosis of cancer be made by examining individual cells

The term "cytologic diagnosis of cancer," assumes that the diagnosis is made by examination of individual cells The majority of authors, however, in their discussions and particularly in illustrations, point to cell masses or groups Several, including Wandall,¹⁶ Papanicolaou,¹⁰ and Dudgeon,²² have pointed to the importance of cell groups Liebow Lindskog and Bloomer²³ stated, "Reliance was placed only upon groups of cells possessing an arrangement suggesting that of tissue, not merely upon individual atypical cells" Albers, McDonald and Thompson²⁴ considered cell clumps essential for the diagnosis of well differentiated carcinoma of the prostate on the basis of examination of prostatic secretions

20 Herbut, P. A., and Clerf, L. H. *J A M A* **130** 1006, 1946 Dudgeon and Wrigley¹¹ Wandall¹⁶ Farber and others¹⁷

21 Diggs, L. W. *Am J Clin Path* **18** 293, 1948

22 Dudgeon, L. S. *St Thomas's Hosp Rep* **1** 51, 1946

23 Liebow, A. A., Lindskog, G. E., and Bloomer, W. E. *Cancer* **1** 223, 1948

24 Albers, D. D., McDonald, J. R., and Thompson, G. J. *J A M A* **139** 299, 1949

It has been emphasized by several authors that mitoses are rarely seen in smear preparations and that they are, therefore, not of great importance. In blocking and sectioning the material, we have observed them frequently in the cell groups and regard them of importance, just as in any other type of biopsy.

It is recommended that special attention be given to the reports of false positive diagnoses. Among these, squamous metaplasia, as emphasized by Wandall,¹⁶ ranks high as a source of error. Some of the specific criteria of cancer must also be present.

Two recent cases in which we have made positive diagnoses, one of which was thought to represent our first false positive diagnosis, have gone on to present the clinical and roentgenographic pictures of cancer. In the first case the lesion was considered inoperable at the time of surgical intervention and a single biopsy revealed a nonspecific granulomatous lesion. In the second case a tuberculoma was removed and was thought to be the only lesion. These cases are not considered entirely proved or disproved and therefore are not included in our statistics. Although tubercle bacilli were not demonstrated in these cases, there may be represented here the coexistence of tuberculosis and neoplasms which Bergmann, Shatz and Flance²⁵ discussed and which was observed by us at autopsy in 2 recent cases.

Thus there have been no proved false positive diagnoses in our experience.

PROCEDURE

It is our practice in each case in which carcinoma of the lung is suspected to review the history, the physical findings and the roentgenograms. The patient is examined with the bronchoscope, and if lesions are seen, specimens are taken for biopsy, and in all cases aspirations and/or washings are obtained, regardless of other findings or procedures.

The material is immediately rinsed with solution of formaldehyde U S P from the vial in which it was collected. It is then centrifuged at 1,000 to 2,000 revolutions per minute for ten to forty-five minutes. After a minimum of four hours' fixation, the supernatant fluid is carefully decanted, and the remaining button wrapped in filter paper if necessary. It is then dehydrated and embedded as any other surgical specimen. Sections are cut from the block and stained with hematoxylin and eosin. This procedure has been used in all cases herein reported.

Several features of this procedure should be emphasized. They have all been encountered, and their neglect has contributed much to the relatively poor results obtained in the early work. The section of tubing connecting the aspirator used with the vial should be washed in order to obtain the material therein. The material should not be centrifugated faster than 2,000 revolutions per minute or for more than forty-five minutes, to avoid laking of the cells. The material must be fixed promptly. The hematoxylin must be destained in water to a point at which nuclear details are distinct.

We have recently adopted the policy of cutting four sections from four different levels because this will increase the likelihood of finding cancerous tissue, especially if the material has been centrifuged

Hunter and Richardson¹ recommended that the material be fixed in a saturated solution of trinitrophenol (picric acid), and the cell mass separated by filtration. These cell masses may be easier to dehydrate and embed, presumably because of greater size and cohesiveness, but we have had insufficient experience with this method to evaluate it fully. Kraushaar and Bradbury²⁶ have reported the use of celloidin (pyroxylin) tips on centrifuge tubes which can be detached and embedded without disturbing the cell mass. Birge, McMullen and Davis²⁷ have recommended the use of powdered fibrinogen and thrombin to coagulate the sediment obtained by centrifugation.

Inasmuch as we examine all suspected patients with the bronchoscope, we have confined ourselves to the examination of aspirated material. In comparative studies, Herbut and Clerf²⁰ emphasized that bronchial secretions were superior to sputum as a source of carcinoma cells, and Woolner and McDonald²⁸ found the two materials equally satisfactory. Aspirated material is delivered as are other surgical specimens to the laboratory, and after centrifugation they are handled exactly as are other surgical specimens.

The professional staff's examination is conducted as with other surgical specimens, with discussion when indicated. Residents become acquainted with the material. Hunter and Fremont-Smith, in their discussion of the report of Pollard, Bryant, Block and Hall,²⁹ who diagnosed gastric neoplasms by cytologic examination of gastric secretions, pointed to the advantages of sections in those examinations, and Hunter stated, "It is my opinion that not until something better than the smear, namely, treating material as tissue, is adopted, will pathologists give any serious consideration to this method for the diagnosis of cancer."

The time and the care required of both technicians and pathologist have been great deterrents to the examination of smear preparations of sputum in the case of general pathologists. Farber and others¹⁷ stated, "It is desirable that at least three to five slides be made from each of five daily specimens, and examined, before a report is given. At least 15 minutes per slide is required by our technicians. Suspicious slides require more time." If five slides are prepared each time and the recommended time spent on each examination, six hours and fifteen minutes per case would be consumed. That does not include the time required of the technician for preparation of the slides or that of the pathologist for final evaluation and diagnosis. As often recommended, the time required per patient may amount to seven or eight man-hours. This is prohibitive in the smaller hospital with a limited staff.

COMMENT

Several discussions dealing with the cytologic diagnosis of cancer, i. e., with the examination of stained smears of fluids which possibly contain exfoliated cancer cells, consider the appearance of individual cells. They also emphasize the great amount of training, experience

26 Kraushaar, O. F., and Bradbury, J. T. *J. Lab. & Clin. Med.* **33** 1195, 1948.

27 Birge, R. F., McMullen, T., and Davis, S. K. *Am. J. Clin. Path.* **18** 754, 1948.

28 Woolner, L. B., and McDonald, J. R. *J. A. M. A.* **139** 497, 1949.

29 Pollard, H. M., Bryant, H. C., Block, M., and Hall, W. C. *J. A. M. A.* **139** 71, 1949.

and time required for the successful use of this method of diagnosis Griffin³⁰ stressed that point. Fremont-Smith, Graham and Meigs³¹ stated, "The ability to make accurate diagnoses by this method is difficult to acquire, obtainable only by months of intensive training and perfected only through constant use." Caution is expressed lest workers lacking the broad training and experience cited attempt to employ this method.

With these statements and cautions we have no argument, we agree that they are basically sound. We note, however, that increasing numbers cite their experience with technics more closely resembling those used in the general practice of pathology. Hunter and Richardson¹ have emphasized the advantages of blocking and sectioning this material. In the procedure we have outlined, no part of the diagnosis is relegated to technicians or "cytologists." Some experience is necessary for the pathologist to assemble the facts with which he is already familiar, to correlate them with the material presented in current literature and to apply the criteria of cancer to this method of examination. For this experience the method of McKay, Ware, Atwood and Harken,¹⁸ in which material removed from the bronchi at the time of autopsy in cases of bronchogenic carcinoma was examined and compared with prepared sections, is to be commended. We believe that by previous training in oncology the pathologist is already basically prepared for this procedure, that by this method no part of the diagnosis is relegated to those with less training and that it is in no way prohibitive economically. Recent reviews of our preparations and of reports made during the past two years and follow-up studies of cases impress us with the fact that we were unnecessarily cautious over a long period.

RESULTS

During a period of twenty-seven months 101 specimens obtained from 81 patients were examined by this method, providing 125 examinations (blocks) per patient. Of this group, 24 have been proved to have carcinoma of the bronchi by biopsy of the primary lesion, surgical resection of tissues for biopsy or of the lung for examination, autopsy or by roentgenogram and clinical progress acceptable to all departments concerned as diagnostic of bronchogenic carcinoma. Of these 24 proved cases of bronchogenic carcinoma, bronchial washings were reported suggestive but not diagnostic in 2, strongly suggestive in 3 and diagnostic of cancer in 10. No diagnosis of cancer was reported in 9 cases.

Nine of the last 10 cases of bronchial carcinoma examined have been positively diagnosed by this method.

30 Griffin, H. K. *Am J Clin Path* 18:330, 1948.

31 Fremont-Smith, M., Graham, R. M., and Meigs, J. V. *J A M A* 138:469, 1948.

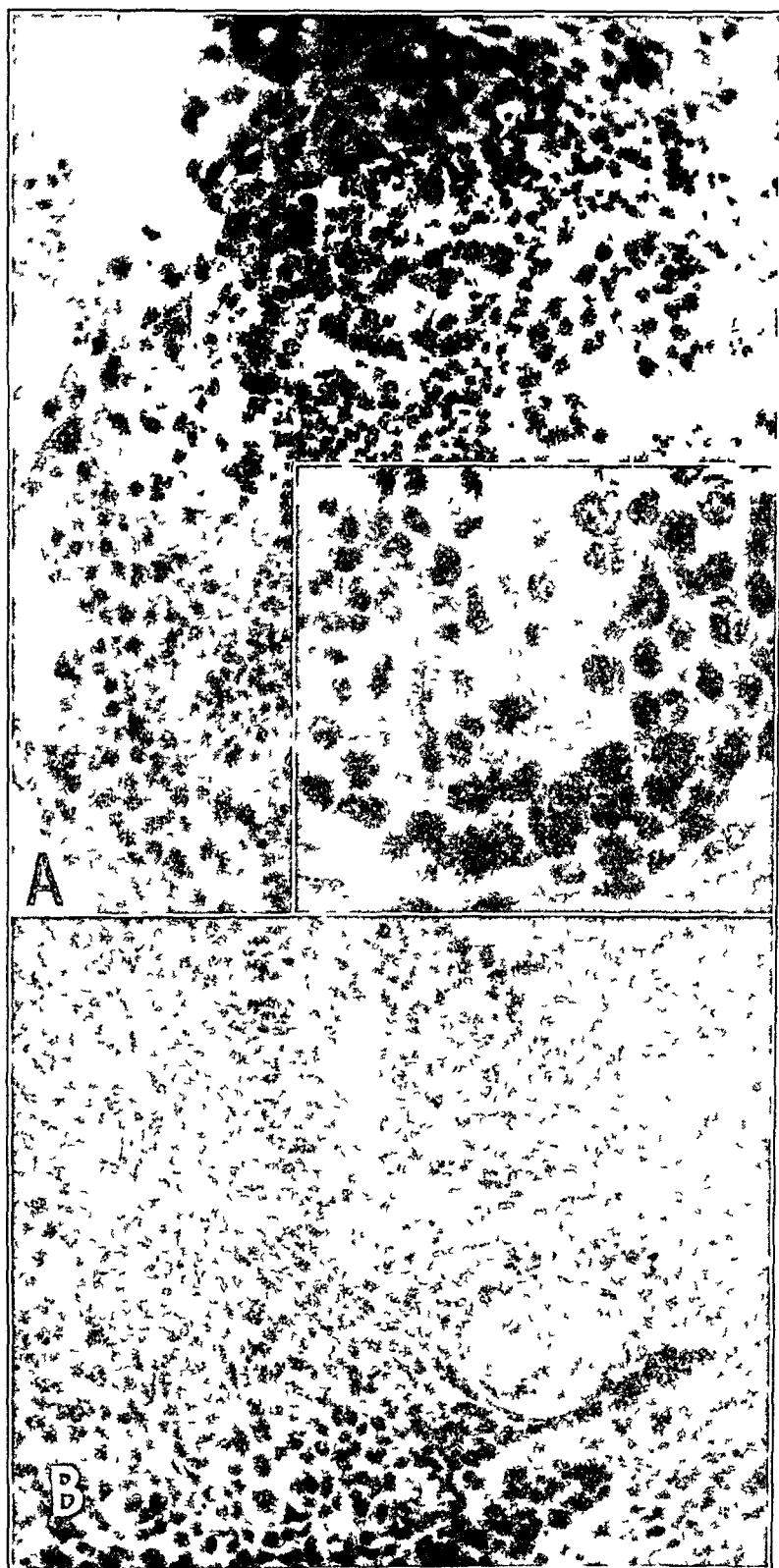


Fig 1 (case 2) —*A*, section of a bronchial washing showing a group of neoplastic cells. Hematoxylin and eosin, $\times 200$. The insert shows loss of polarity and variations in nuclear size. A mitotic figure is seen at lower center. $\times 700$. *B*, squamous carcinoma of bronchus. Hematoxylin and eosin, $\times 200$.

REPORT OF CASES

CASE 1—A 48 year old white man entered this hospital because of pain in the left upper region of the chest and hemoptysis of six days' duration. Roentgen examination revealed a homogeneous dense lesion in the upper lobe of the left lung. The sputum contained no acid-fast bacilli. Bronchoscopic examination failed to disclose a neoplasm. Bronchial washings showed cell groups which were suspected of being carcinoma. The clinical findings were not considered diagnostic of cancer, but the lesion persisted, and repeated roentgen studies, including angiography, contributed suggestive but not conclusive evidence of a neoplasm. Thoracotomy was offered but refused, and the patient was discharged, only to be readmitted three months later with essentially the same complaints. The lesion had not diminished in size, as shown by roentgenogram. A biopsy gave negative results, but bronchial washings again showed cell groups which aroused a suspicion of carcinoma. The left lung was resected, and a large neoplasm limited to the upper lobe was found. The pathologic diagnosis was bronchogenic carcinoma, squamous cell type.

CASE 2—A 50 year old white man was admitted to the hospital, complaining of cough and pain in the chest of four weeks' duration. Roentgenograms of the chest revealed an infiltrative process, thought to be inflammatory, in the upper lobe of the right lung. The sputum contained no acid-fast bacilli or fungi. Bronchoscopic examination revealed a few raised areas at the orifice of the bronchus of the upper lobe of the right lung. "They did not have the appearance of a tumor, but did bleed slightly on manipulation." A biopsy was not made. Histologic sections of bronchial washings were positive for carcinoma (fig 1 A). Pneumonectomy was performed on the right, and a primary neoplasm, which measured 5 cm in diameter, was found in the upper lobe of the right lung. Histologic sections showed it to be a moderately well differentiated squamous cell carcinoma (fig 1 B). The patient died ten weeks after operation. At autopsy no metastases were found.

CASE 3—A 53 year old white man was admitted for the diagnosis of a lesion which had been found in the left upper lung field, on routine roentgen examination of the chest at another hospital. He had no symptoms referable to the lungs. There was dullness to percussion over the left upper lung field with diminution of tactile and vocal fremitus and distant breath sounds. Bronchoscopic examination revealed narrowing of the left main bronchus, thought to be due to an extrinsic lesion of the upper lobe. No endobronchial lesion was seen, and no specimen was obtained for biopsy. The bronchial washings were considered diagnostic of carcinoma. The left lung was resected, and a large primary neoplasm was found in the upper lobe. The pathologic diagnosis was adenocarcinoma of the bronchus of the left upper lobe.

CASE 4—A 50 year old white man was admitted for the fifth time because of a "brassy" cough of several years' duration and recurrent pain in the anterior part of the chest. Roentgen examination revealed that the heart and lungs were within normal limits. The bronchoscopic diagnosis was "asthmatic tracheobronchitis, severe." Prepared bronchial washings yielded many large groups of carcinoma cells in sections (fig 2 A). Further roentgen examinations of the chest and planigraphic studies of the trachea and the main bronchi failed to reveal evidence of an intrinsic or an extrinsic lesion. Additional bronchoscopic examinations made over a period of two months finally showed a slight heaping up of tissue with narrowing of the lumen of the right main bronchus. Biopsy of this tissue revealed a poorly differentiated carcinoma (fig 2 B). Pneumonectomy was done promptly on the right side (fig 3) and histologic sections of the right main bronchus revealed an early primary squamous cell carcinoma with peribronchial extension only.



Fig 2 (case 4) —*A*, sections of bronchial washings of neoplastic tissue $\times 200$
B, biopsy specimen showing poorly differentiated carcinoma Hematoxylin and eosin, $\times 200$

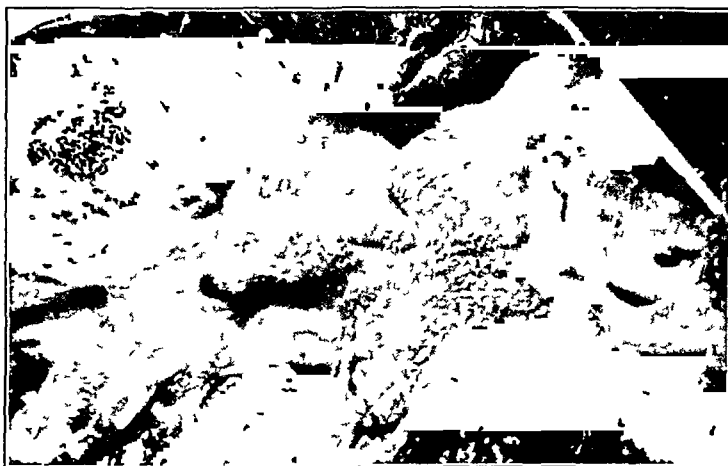


Fig 3 (case 4) —Squamous carcinoma of the right main bronchus A stick has been placed along the line of resection Note that there are thickening and granularity for a distance of 5 cm in the center of the photograph

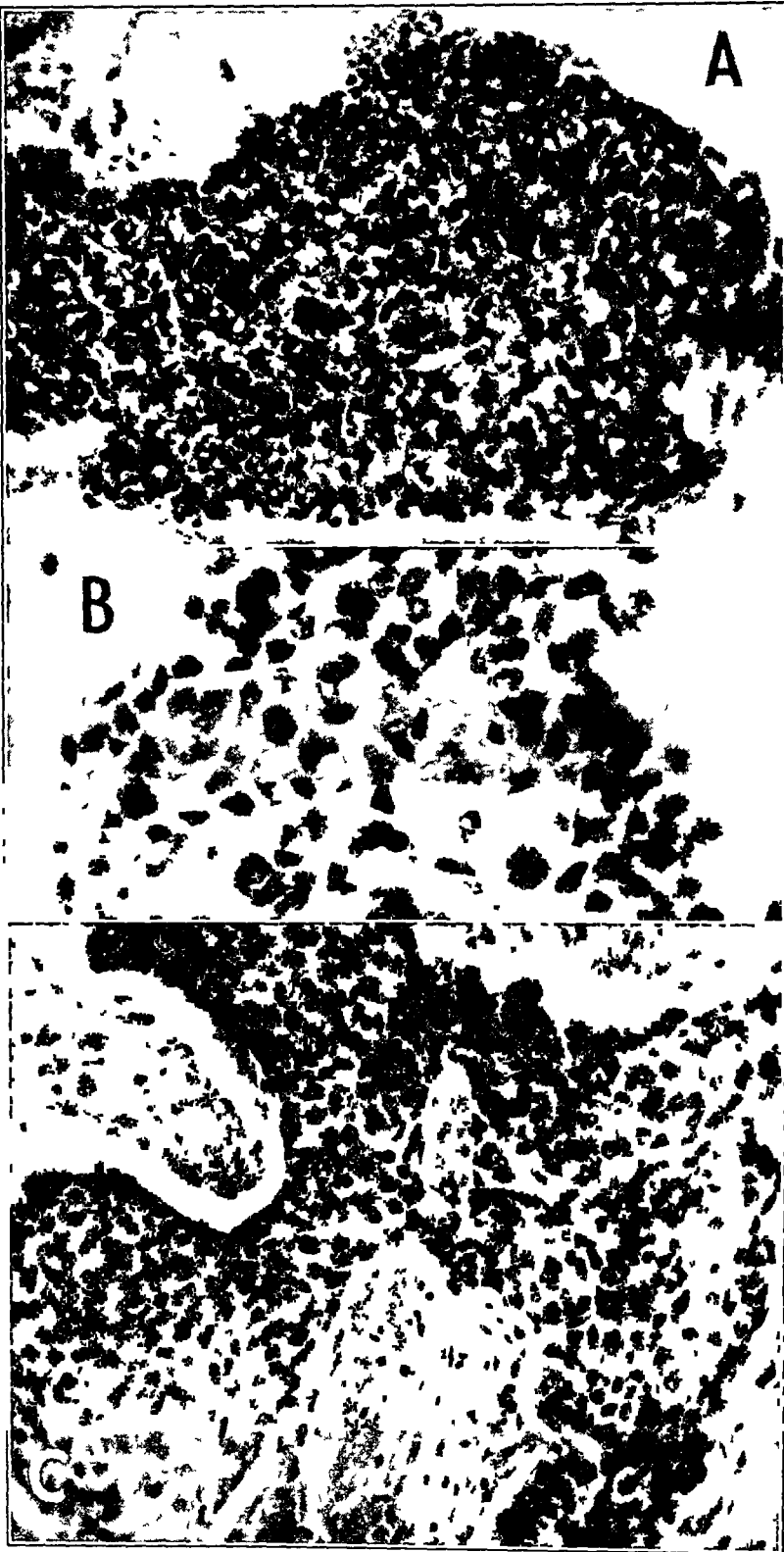


Fig 4 (case 5) —*A*, section of bronchial washing containing a large island of neoplastic tissue. Hematoxylin and eosin, $\times 200$. *B*, field from *A* showing loss of polarity, variations in size, contours and staining intensity of nuclei, and mitoses. Hematoxylin and eosin, $\times 700$. *C*, section of squamous carcinoma of the bronchus of the upper lobe of the right lung, found at autopsy. Hematoxylin and eosin, $\times 350$.

CASE 5—A 60 year old white man complained of painful joints and varicose veins. The roentgenogram presented a right hilar shadow which was interpreted as probably bronchogenic carcinoma. On bronchoscopic examination a mass was observed in the bronchus of the upper lobe of the right lung. Because of its position, a good biopsy specimen could not be obtained. Sections of the bronchial washings were considered extremely suggestive of cancer (figs 4 *A* and *B*). Pathologic examination of the tissue revealed bronchial mucosa that was reported to be suggestive, but not diagnostic of cancer. Exploratory thoracotomy was performed and an inoperable lesion of the upper lobe of the right lung was found. At autopsy, histologic sections of the lesions of the right upper lobe bronchus revealed a primary, poorly differentiated epidermoid carcinoma (fig 4 *C*).

It is of interest to note that this case was seen early in our series. Caution at that time is illustrated in the diagnosis of the aspirated material as suggestive of cancer.

SUMMARY

Follow-up studies to date demonstrate that our policy and procedure are effective in that carcinoma of the bronchus has been detected early in a reasonably high percentage of cases. Our failures to identify cancer cells occurred principally during the first half of the period reported. A review of our past material at this time indicates that skepticism as to the value of the procedure and unnecessary caution not only lowered the percentage of diagnoses but delayed appreciation and adoption of the procedure as a valuable diagnostic aid.

Aspirated material and/or washings obtained through the bronchoscope constitute a valuable source of cancer cells, particularly as cell groups, for the diagnosis of bronchogenic carcinoma. This material, after centrifugation, can be fixed, embedded, sectioned and stained just as any other biopsy material. From our experience and a review of current literature we conclude that the examination of aspirated bronchial material and/or washings constitutes an aid to the early diagnosis of bronchogenic carcinoma of such great value that its use can no longer be ignored by the general pathologist.

ACCLIMATIZATION RESPONSE AND PATHOLOGIC CHANGES IN RATS AT AN ALTITUDE OF 25,000 FEET

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AND

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A STUDY of the growth and reproduction of rats exposed to a high altitude for four hours daily has been reported by one of us (Altland¹). In the course of that study it was found, as will be reported here, that such exposures produced certain acclimatization responses, often severe changes in the heart and other organs, and premature death. Other investigators² have studied the organic changes associated with acute high altitude hypoxia and short term continuous hypoxia, but the nature of the physiologic adjustments and the severity of the pathologic changes encountered in these long term experiments have not been reported previously.

METHODS

The procedure of exposure was essentially the same as that previously described¹. One hundred and seventy-nine male and 130 female Sprague-Dawley rats were exposed to 25,000 feet of simulated altitude four hours daily, starting at 14 days of age. Of these, 79 males and 60 females were killed for study, 59 males and 36 females died in the altitude chamber during exposure, and 41 males and 34 females were found dead in their cages during intervals of rest. All rats were examined as soon as possible, usually within four hours after death, but a delay of as long as twelve to eighteen hours was unavoidable with some rats that died at night.

The tissues saved for histologic examination were generally fixed in a buffered solution of formaldehyde. The bones were decalcified with 5 per cent formic acid. Paraffin sections were stained routinely with azure eosinate,³ with the

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1 Altland, P D. *J Exper Zool* **110** 1 1949.

2 Campbell, J A. (a) *J Physiol* **62** 211 1927, (b) *ibid* **63** 325, 1927, (c) *Brit J Exper Path* **16** 39, 1935. (d) Armstrong, H G, and Heim, J W. *J Aviation Med* **9** 45, 1938. (e) Sundstroem, E S, and Michaels, G. *Univ California Publ* **12** 1, 1942. (f) Dalton, A J, Jones, B F, Peters, V B, and Mitchell, E R, *J Nat Cancer Inst* **6** 161, 1945. (g) Reynolds, O, and Phillips, N E. *Am J Physiol* **151** 147, 1947. (h) Lewis, R B, and Haymaker, W. *J Aviation Med* **19** 306, 1948.

3 Lillie, R D. *Histopathologic Technic*, Philadelphia, The Blakiston Company, 1948.

acidulated ferrocyanide reaction for iron and with the Dunn-Thompson modification of Van Gieson's stain for hemoglobin,⁴ and occasionally, when indicated, with Masson's trichrome and other special stains. Frozen sections of heart, liver, kidney and adrenal gland were stained for fat with oil red O.⁵

The organ weights of exposed rats killed for study were compared with those of control rats of equivalent body weight. This was necessary since the exposed female and male rats weighed 21 and 36 per cent less than controls of corresponding ages.¹

Tail blood samples were obtained at regular intervals and analyzed for percentage of packed red cells and for hemoglobin content. The hematocrit value was obtained by using Van Allen tubes and centrifuging at 2,000 revolutions per minute for thirty minutes. Oxyhemoglobin concentration was determined

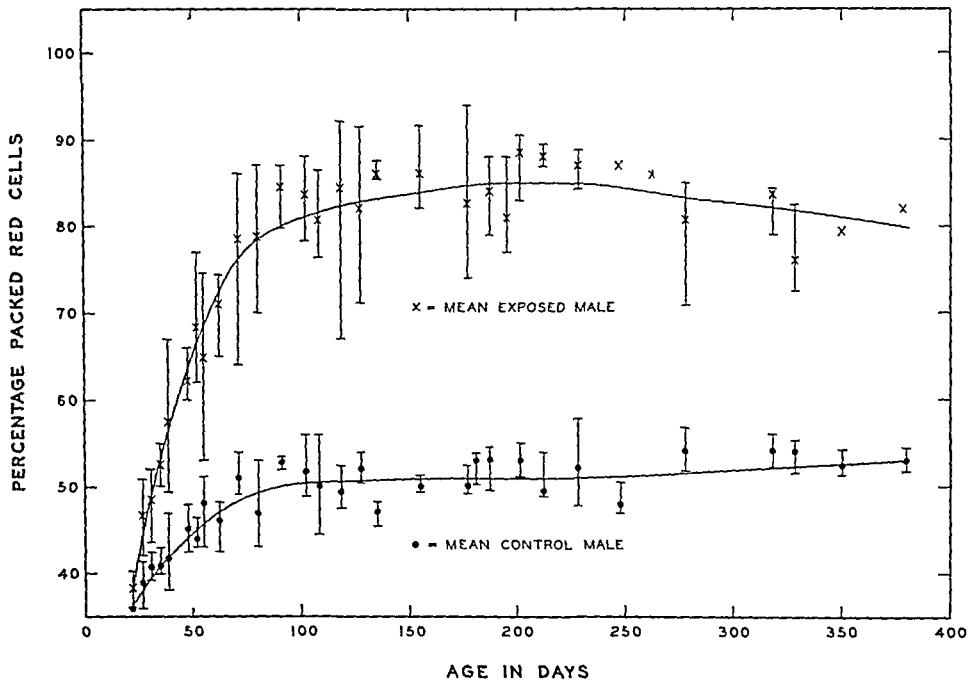


Fig 1—Hematocrit values of male rats exposed four hours daily to a simulated altitude of 25,000 feet. Maximum and minimum values are given for various ages.

with a Beckman spectrophotometer by using extinction coefficients reported by Horecker.⁶ Measurements were taken at wavelengths of 5100, 5400, 5600 and 5765 angstroms to establish whether any pigments other than oxyhemoglobin were present. An average value of these four readings was used to calculate the percentage of oxyhemoglobin.

RESULTS

An important effect of the altitude to which the rats were daily exposed was the development of polycythemia. The hematocrit and oxyhemoglobin values

4 Dunn, R. C., and Thompson, E. C. *Arch Path* **39** 49, 1945.

5 Lillie, R. D., and Ashburn, L. L. *Arch Path* **36** 432, 1943.

6 Horecker, B. L. *J Biol Chem* **148** 173, 1943.

of both sexes began to increase gradually during the second week of exposure and continued to rise until a plateau was reached between 70 and 100 days (fig 1 and 2) Although there was some individual variation, the mean values of the hematocrit and the oxyhemoglobin concentration were 39 and 35 per cent, respectively, above control levels It is noteworthy that these high levels persisted for the duration of the experiment in most of the rats which maintained their body weight The mean count for 20 exposed rats over 90 days old was 10,235,000 erythrocytes per cubic millimeter (range, 8,500,000 to 11,200,000) while that of a similar number of controls was 8,000,000 (range, 7,550,000 to 8,450,000) The leukocyte count of these exposed rats was not increased Analysis of smears of the femoral marrow of 10 of the exposed rats showed an absolute increase of the precursors of erythrocytes and an absolute decrease of those of leukocytes (The data on the marrow smears and the blood counts of these rats were fur-

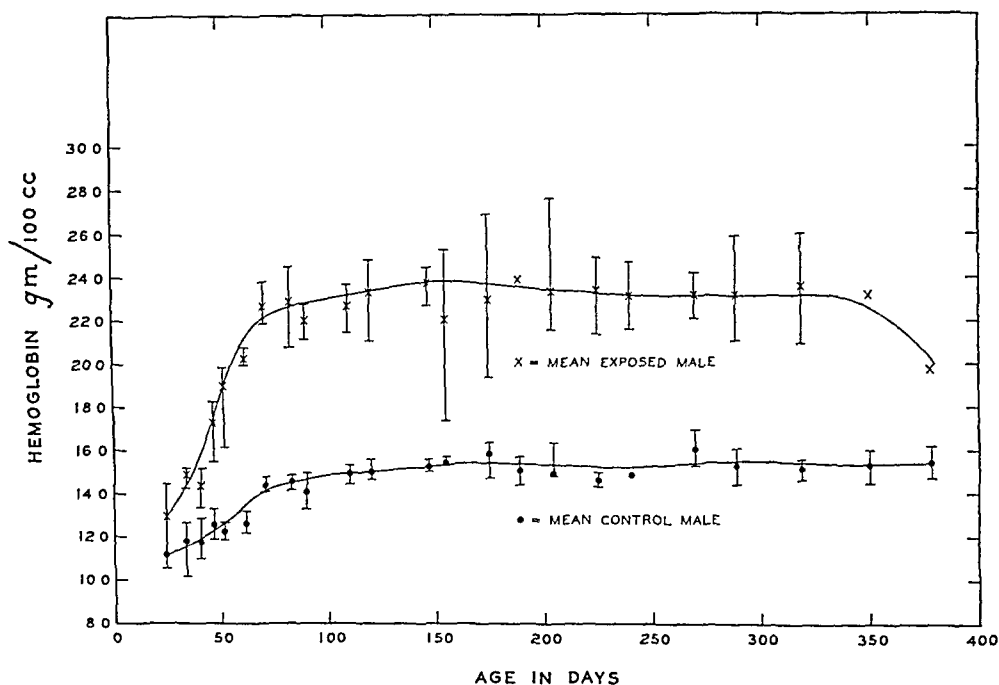


Fig 2—Hemoglobin concentrations of male rats exposed four hours daily to a simulated altitude of 25,000 feet Maximum and minimum values are given for various ages

nished by Dr K M Endicott) The number of megakaryocytes seen in sections of the marrow was not significantly altered Thrombocyte counts were made on 6 additional exposed rats and 5 controls The counts of 3 exposed animals were normal and those of the other 3 were decreased All 6 exposed animals showed polycythemia

The ability of the animals to withstand daily exposure to a simulated altitude of 25,000 feet varied greatly Approximately 30 per cent of the rats died during the first 100 days, 65 per cent were dead by the end of 200 days, and only 10 per cent survived more than 300 days There was little difference in the ability to withstand the stress between the males and the females The oldest female reached 433 days of age and the oldest male 400 days, or less than half of the normal life span of this strain of rats Some rats died unexpectedly, usually in the altitude chamber, without warning signs or symptoms, in other instances

death was preceded by a reduction of the activity of the rat associated with a loss of body weight and often a drop of blood values

PATHOLOGIC OBSERVATIONS

Altogether, the tissues of 38 control rats (none\posed), 47 exposed rats killed for study and 61 exposed rats that died during the experiment were studied histologically and comparisons made according to age and sex. Pathologic changes occurred in all the exposed rats, but severe changes were generally more common in the exposed rats that died than in those that were killed. One of the most constant findings in exposed rats was gross hypertrophy of the heart, with an increase of its weight. There was considerable individual variation in the relative

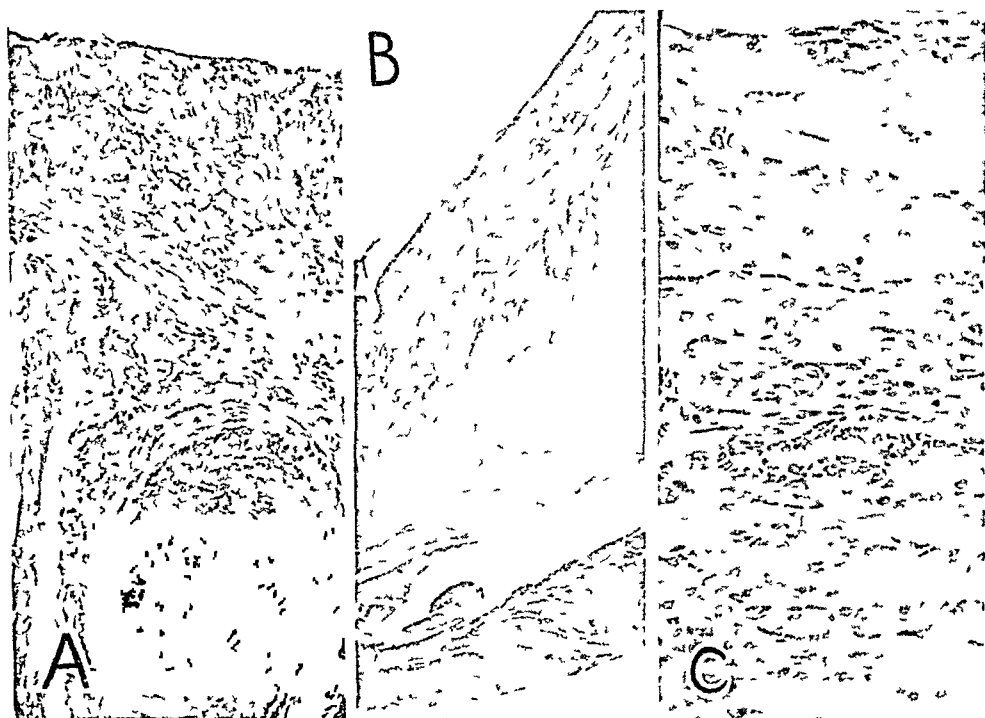


Fig 3—*A*, section of the heart of a rat 119 days old, showing extensive infarction of the myocardium with a prominent inflammatory reaction. Note the organizing thrombus in the large artery lying between the atrium and the right ventricle. The epicardium is in the upper portion of the figure. Masson's trichrome stain, $\times 70$.

B, section of the heart of a rat 101 days old, showing fibrous changes in the myocardium around the left ventricle but not around the right ventricle. Azure eosinate, $\times 12$.

C, higher power view of area of left ventricle shown in *B*. Note preservation of muscle fibers immediately beneath the endocardium. Azure eosinate, $\times 200$.

gain in cardiac weight but there was no significant difference in this respect between the sexes or between the age groups. The chambers were frequently dilated and their walls thickened. The average diameter of 20 or more fibers in the interventricular septum was determined and was found to exceed 14 microns in 52 of 92 exposed rats and in only 2 of 36 controls.

One male rat found dead at 119 days had infarction of most of the myocardium and organizing thrombi in two large and several small arteries at the base of the heart (fig 3 *A*). A male rat dying at 168 days showed subtotal scar tissue replacement of the interventricular septum. In both of these rats there were vegetations on the aortic valve (described in a later paragraph). Six additional rats showed areas containing many scattered, poorly staining, atrophic or necrotic muscle fibers. Slight myocardial fatty degeneration was seen in 8 of 42 males and in 1 of 25 females over 100 days of age, and several rats had small hemorrhages in the myocardium. In about half of the exposed rats there was a relative increase in the fibrous tissue with disappearance of many muscle fibers in the inner third of the myocardium surrounding the left ventricle (fig 3 *B*). This occurred less frequently in the septum and near the apex and occasionally elsewhere. Generally such areas showed numerous fibroblasts and mononuclear cells, often a few phagocytes laden with fat or hemosiderin, and occasionally a few atrophic, fatty or frankly necrotic muscle fibers. The muscle fibers immediately beneath the endocardium were usually preserved (fig 3 *C*).

The endocardial changes were confined chiefly to the valves (fig 4). The majority of the exposed rats had slight to marked irregular or nodular thickening of the valves, usually most impressive in the distal portion. The changes were more frequent and severe in the mitral than in the aortic and tricuspid valves. The maximum thickness of some valves was more than five times that of the controls. The thickened valves often showed marked cellular proliferation and focal areas of edema, mucoid degeneration, hemorrhage and, chiefly in older rats, fibrosis, hyalinization, necrosis or calcification. Capillaries were numerous in some of the thickened valves and occasionally extended to the free edge. Organizing vegetations of variable size were seen along many of the thickened mitral and aortic cusps and on the cusps of one tricuspid valve. The incidence increased with age, vegetations were seen in only 1 rat under 100 days of age and in about half of the rats over 300 days. They occurred chiefly near the line of closure and along the upper surfaces of the valves. Phagocytes laden with brown pigment, chiefly hemosiderin, were seen submarginally in some valves, and fat-laden phagocytes were frequent around necrotic foci and at the base of vegetations. Some vegetations were partially covered by endothelium, a few contained small groups of neutrophilic leukocytes, and a few showed occasional basophilic granules and small deposits of calcium. The mitral valve of a rat that died at 349 days of age was densely infiltrated by neutrophils in some areas and showed many large colonies of gram-positive and gram-negative micro-organisms along the borders of a large vegetation. No organisms were found in other tissues of this rat. A similar vegetation with bacterial colonies was seen on the tricuspid valve of a 433 day rat. Gram and fibrin stains made on the cardiac valves of a number of other rats revealed fibrin but no bacteria in the vegetations.

The auricular endocardium occasionally showed focally slight cellular proliferation, fibrous thickening, ulceration or calcification. The auricles of 6 of 60 rats contained unorganized and organized vegetations, some of these showing a little hemosiderin and calcium.

The epicardium of a few animals was thickened and moderately infiltrated by monocytes. This condition was usually associated with either pleuritis or some adjoining myocardial lesion. Two rats showed epicardial hemorrhages overlying necrotic areas.

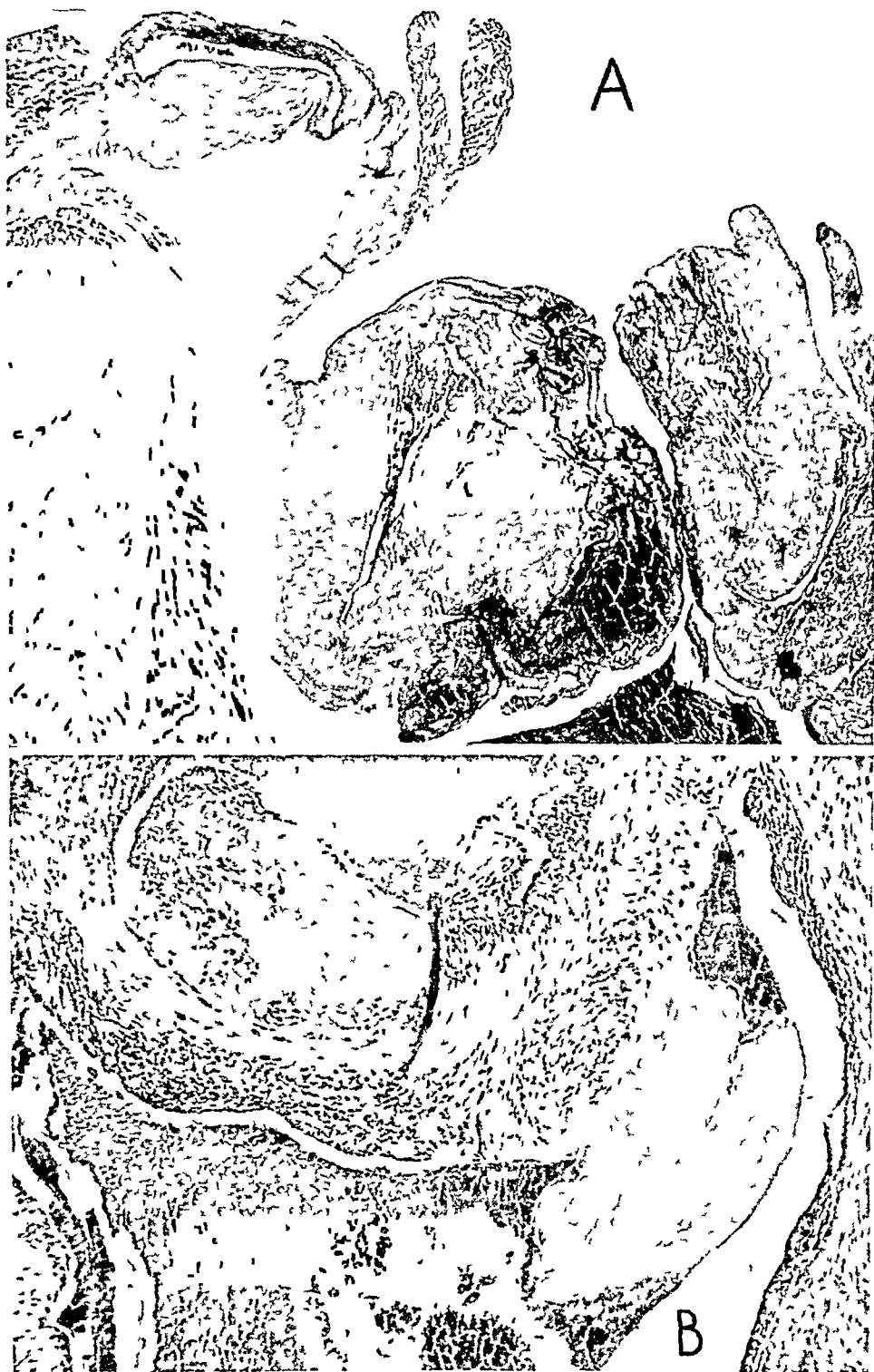


Fig 4—*A*, section of the heart of a rat 117 days old, showing distinct thickening of the mitral valve with large vegetation Van Gieson stain as modified by Dunn and Thompson, $\times 20$

B, Section of the heart of a rat 309 days old, showing vegetations on the base of the aortic valve Note that the upper vegetation is organized and partially covered by endothelium Azure eosinate, $\times 50$

There was considerable variation in the weight of kidneys of exposed rats. Nearly all showed notable capillary congestion. The average diameter of the glomeruli, estimated by measuring 20 to 50 in each kidney, exceeded 130 microns in 49 of 95 exposed rats and in only 4 of 34 unexposed controls. Since tangential sections of glomeruli were included, the actual average diameter is higher than the estimated average.

Nineteen of 80 rats over 100 days old and one 60 day rat showed single or multiple infarcts (fig 5A) of one or both kidneys, occasionally involving nearly an entire kidney. There was no striking difference in incidence between the sexes and different ages. The infarcts appeared triangular in sections with a thin subcapsular layer of viable parenchyma along the base. Nearly all were of the anemic type, but a few were hemorrhagic throughout or showed a hemorrhagic border, sometimes infiltrated focally by neutrophils. In older rats the infarcts often showed tubular and glomerular atrophy, extensive fibrosis, hyalinization and calcification, and definite shrinkage with depression of the overlying capsular surface. In a few of the kidneys there were small wedge-shaped cortical scars suggesting healed infarcts. Several kidneys had thrombosed vessels in infarcted areas. One showed recanalization of a thrombus in a medium-sized artery in a noninfarcted area, and one had a thrombotic mass, possibly an embolus, in a small artery near an infarct. Numerous neutrophils, but no bacteria, were seen in the thrombus and in and around the vessel wall. Another kidney, with a recent and a focally calcified old infarct, had a similar clot in a large pelvic artery at a point where the lumen was narrowed by a large hemosiderotic fibrotic nodule. This nodule resembled an old organized thrombus or embolus. The renal infarcts were often associated with thickening and vegetations of the valves of the heart. Many of the infarcted kidneys also showed slight to moderately extensive focal or segmental fatty changes in the muscular coat of the large and medium-sized arteries (fig 5C). Such changes were occasionally present in noninfarcted kidneys but were not seen in the heart, the liver or the adrenal glands.

Marked hemosiderosis of the renal cortex was seen in nearly all rats after ten weeks' exposure. Heavy deposits were seen along the borders of infarcts and in scattered clusters of convoluted tubules, and lesser amounts in intervening tubules. The pigment granules were most prominent at the base of the epithelial cells and ranged in size from barely visible to more than 5 microns. They were seen occasionally in intratubular desquamated cells, in fibroblasts and capillary endothelial cells and rarely in the epithelium of medullary tubules.

Small numbers of scattered tubular hemoglobin casts (usually less than 10 per section) were frequent in both control and exposed rats. Numerous hemoglobin casts (from 35 to over 100 per microscopic section) were seen in 9 of 53 exposed rats but in none of 16 controls between 100 and 300 days of age and in 3 of 11 control rats over 300 days. In addition, some exposed rats showed hyaline casts and pigmented casts, some of the casts staining in part like hemoglobin.

About 33 per cent of the males and 46 per cent of the females over 100 days had a slight to moderate increase of lipid material in the kidney. The lipid material was seen chiefly in the epithelium of scattered small groups of cortical tubules and along the borders of infarcts. Several kidneys disclosed fibrosis, hyalinization and less severe changes in many glomeruli and epithelial desquamation, hyaline droplets and other changes in many tubules. Variable numbers of small calcareous deposits were seen in several exposed and 1 control animal, chiefly at the junction of cortex and medulla, and often gave the prussian blue reaction for iron.

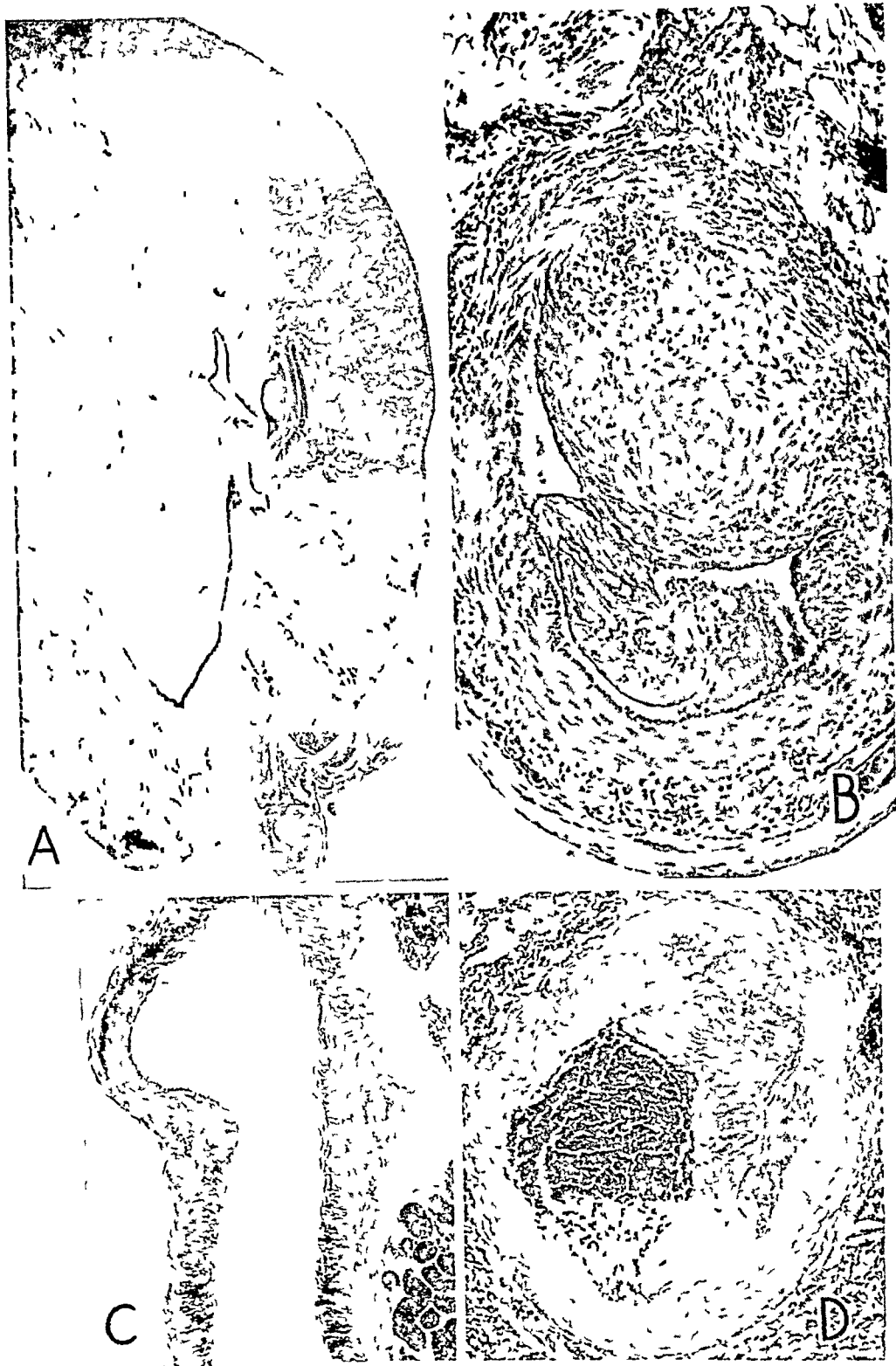


Fig 5—*A*, section of a kidney of a rat 309 days old, showing a large recent infarct above, a smaller, older infarct below and a thrombosed artery at the bottom of the section Azure eosinate, $\times 10$

B, higher power view of the artery in *A* Note the fibrous nodule above and the recent thrombus below, free in the lumen of the artery Azure eosinate, $\times 100$

C, frozen section of a kidney of a rat 298 days old Note focal fatty changes (dark areas) in the media of the large artery Many tubules show dark fat droplets and lighter (gray) hemosiderin granules Oil red O, $\times 60$

D, section of a lung of a rat 332 days old, showing a large vessel with extensive fibrous thickening and focal calcification of the intima and a large subendothelial fibrinous plaque Masson's trichrome stain, $\times 15$

Gross hematuria, usually confirmed microscopically, was observed in 6 males and 7 females between 79 and 226 days of age. Ten of these rats died shortly thereafter. In 1 of the 3 that survived, the hematuria was followed by a temporary sharp drop in blood count, hematocrit reading and hemoglobin value, and autopsy 177 days later revealed an old renal infarct. The 2 other rats that survived after hematuria later revealed small hemosiderotic cortical scars suggesting healed infarcts. The kidneys of 4 rats with terminal hematuria were studied histologically. All had distinct congestion and hyaline droplets or other severe degenerative changes in the renal convoluted tubules, 3 showed focal fatty changes in the media of some of the larger blood vessels, 1 a recent thrombus in a large artery, and 1 an area suggesting early infarction.

The average weight of the spleens of rats exposed over 105 days was 24 per cent more than that of the controls. Erythropoiesis was on the whole much more appreciable in the spleens of exposed rats under 200 days than in those of controls of a similar age. Hemosiderin was greatly reduced in amount or absent in nearly all rats under 100 days, and was relatively small in amount in males thereafter, but approached normal in females over 200 days. Many exposed rats, particularly the males, also showed a slight to marked decrease in the average size of the malpighian corpuscles and in the number and the size of the germinal centers. The perifollicular zone of pale reticulum cells was often narrowed and poorly demarcated.

Controllobular congestion with narrowing of the liver cell cords was seen in nearly all the exposed rats and was severe most frequently in those between 100 and 200 days of age. Slight to moderate hemosiderosis of Kupffer cells, rare in controls, was seen in about 20 per cent of exposed males and 50 per cent of exposed females over 100 days old. About 20 per cent of exposed males and females over 100 days old had slight to moderate fatty changes in the liver cells.

Severe intestinal hemorrhage with massive accumulation of blood occurred in both males and females and was found in 26 per cent of all rats that died during the experiment. In many cases the entire intestine was engorged with blood. The cecum and adjoining gut were most frequently affected. This condition was found after 7 weeks of exposure (age, 65 days) and as late as the forty-ninth week (age, 363 days) but was most prevalent between the third and the ninth month of exposure. Gross examination of the intestinal mucosa revealed no clearly recognizable localized source of the bleeding. The intestines of 8 of these rats were examined microscopically. All showed severe congestion and increased vascularity throughout, with extensive disorganization and hemorrhages in the mucosa. Three showed ulceration of the mucosa, with polymorphonuclear leukocytes infiltrating the subjacent submucosa and, in 2 rats, the muscularis. A few leukocytes were seen focally in other areas.

The stomachs of the rats with blood in the intestinal lumen were without exception devoid of blood. With the dissecting microscope small gastric ulcers were seen in the stomachs of 5 of 79 exposed rats. Histologic study of one of these gastric ulcers revealed an irregular base of dense fibrous tissue covered by fibrinoid material admixed with nuclear debris and a few leukocytes. The muscularis mucosae was interrupted, and the underlying submucosa was markedly thickened and showed numerous capillaries, fibroblasts and mononuclear cells, and occasional hemosiderin-laden phagocytes. A small artery in the submucosa near the ulcer showed marked hyaline thickening of the intima.

The lungs of the exposed animals were often strikingly congested, and the majority showed slight to moderate hemosiderosis of the septal cells and of

occasional alveolar phagocytes In a few rats the hemosiderin was confined to a portion of one lobe, suggesting a previous hemorrhage

A female rat that died at 295 days showed purulent bronchitis and consolidation of one lobe with extensive atelectasis, interalveolar fibrosis and areas of organizing pneumonia and hemorrhage Many alveoli were lined by cuboidal type cells, many were filled with large mononuclear cells heavily laden with hemosiderin, and some contained elongated brown crystals staining like hemoglobin In some hemorrhagic areas the alveolar septums were not clearly seen, suggesting infarction

In the lung of another rat, killed at 332 days, there were numerous large vessels showing marked irregular thickening of the intima (fig 5D) Along the lumen were massive plaques, some partially covered by endothelium, formed of hyaline eosinophilic material staining like fibrin, admixed with nuclear debris and a few scattered mononuclear cells In areas not covered by endothelium the plaques were often margined with leukocytes The intima was formed of moderately cellular, dense fibrous tissue which often showed areas of hyalinization, necrosis and calcification The media was usually thin and frequently scarred In a few vessels it contained striated muscle fibers (pulmonary veins) The adventitia was often inconspicuous Such vascular changes were not seen in the other organs The heart of this rat revealed marked thickening of the mitral valve with organized vegetations in the left auricle

A few rats showed pulmonary emphysema and a few focal calcification of pulmonary vessels, but the incidence of edema, hemorrhages, atelectasis and pneumonia was not significantly increased in the exposed animals

The thymuses of exposed rats weighed from 5 to 77 per cent (mean, 40 per cent) less than those of controls, but the nature of the cyclic development and regression of this gland during the first four months of life makes comparison difficult Nearly all the thymuses studied were markedly congested Thymic hemorrhages occurred frequently in exposed rats under 200 days that were found dead and occasionally in older rats The incidence and the degree of cortical atrophy were relatively greater in exposed rats under 200 days than in controls of similar age In older rats the incidence was about the same

The cervical lymph nodes of both sides of 9 exposed rats 94 days old were removed and weighed wet The mean weight of the pooled nodes was 69 mg (range, 30 to 139 mg), compared with a mean weight of 175 mg (range 170 to 180 mg) for those of controls The lymph nodes varied considerably histologically, some differing little from those of corresponding controls In general, they showed a considerable reduction in the number of primary follicles and in the number and the size of the germinal centers Often the medulla revealed a relative increase in size and loose structure with wide sinusoids, and occasionally it contained fatty tissue Hemorrhagic extravasations and a variable amount of phagocytosed hemosiderin were seen in the nodes of a few older rats found dead after exposure Examination of the vertebrae and the femurs of these 9 exposed rats disclosed slight to considerable congestion of the marrow with increased cellularity and absence of fat The smaller vessels of the meninges and the spinal cord were dilated and engorged

The spinal cords and brains of 15 animals were examined and showed striking vascular engorgement throughout One rat killed for study at 391 days had a tumor of the pons, probably a glioma This tumor is considered to have been an incidental finding, unrelated to the hypoxia, and will be reported in detail in a separate paper

Partial or complete paralysis and cyanosis of the hindlimbs was observed in a male aged 217 days and in 3 females aged 98, 124 and 128 days. These symptoms appeared suddenly, preceded only by a slight loss of weight, and culminated in death from 1 to 4 days. During this period the rats moved about by propelling themselves with their forelimbs. The blood values of these rats remained high, one such rat had a hematocrit reading of 90 per cent and a hemoglobin concentration of 26 Gm per hundred cubic centimeters one day before death. The 217 day old male had blood in the subarachnoid space, the cerebral ventricles and the central canal of the spinal cord. In the 128 day female there was an extradural hemorrhage dorsal to the spinal cord with a slight lymphocytic infiltration of a dorsal ganglion and adjacent spinal nerve roots. This infiltration is considered to have been an incidental finding.

The findings in the reproductive system and the adrenal glands have been detailed elsewhere.⁷ In general, the testes showed premature sloughing of spermatids and spermatocytes, and thus a marked reduction of the number of spermatozoa was effected. These sloughed cells were abundantly present in the lumens of the epididymis. The ovaries showed no structural abnormalities, and cyclic estrus was not usually disturbed, but gestation was affected to such a degree that no living young were produced.

The adrenal glands generally showed slight to distinct congestion, with widening of the sinusoids, particularly in the outer part of the zona reticularis, occasionally grading into necrosis. The cortex was thickened in most exposed rats over 100 days, and in at least some the diameter of the medulla was considerably increased. Slight to moderate hemosiderosis of the vascular endothelium was frequent, particularly in females. The concentration of lipid material was often reduced, particularly in the zona glomerulosa of exposed rats over 300 days old.

COMMENT

Our findings indicate that severe cardiac damage may result from repeated exposure to high altitude. Hypertrophy and fatty degeneration of the heart have been described by other investigators, but the occurrence of infarction, fibrosis and other histologic evidence of severe myocardial damage, such as that reported herein, apparently has not been reported in previous altitude studies.⁸ Thickening of the atrio-ventricular valves has been reported by Dalton and co-workers^{2f} in rats exposed twelve weeks, but the changes were apparently less frequent and severe than in our study. Moreover, he found vegetations only on the mitral valve. As a group, the cardiac vegetations in our rats appeared sterile. The incidence of infected vegetations approximated that of the bacterial endocarditis found by Wilens and Sproul⁹ to occur spontaneously in the rat.

7 Altlund, P. D. *Proc. Pennsylvania Acad. Sc.* **22** 35, 1948, footnote 1.

8 Van Liere, E. J. *Anoxia: Its Effect on the Body*, Chicago, University Chicago Press, 1942. Van Liere, E. J. *Am. J. Physiol.* **116** 290, 1936. Campbell, J. A. *Brit. J. Exper. Path.* **8** 347, 1927. Thorn, G. W., Clinton, J., Jr., Farber, S., and Edmonds, H. W. *Bull. Johns Hopkins Hosp.* **79** 59, 1946. Campbell^{2a}, Armstrong and Heim^{2d}, Sundstroem and Michaels^{2e}, Dalton and others^{2f}, Reynolds and Phillips^{2g}, Lewis and Haymaker^{2h}.

9 Wilens, S. L., and Sproul, E. E. *Am. J. Path.* **14** 177, 1938.

The frequent occurrence of thrombosis, infarction, hemorrhages and peptic ulcer in polycythemia vera¹⁰ (primary polycythemia) suggests that secondary polycythemia may have been responsible in part for similar findings in our animals. However, in contrast to the usual findings in primary polycythemia, our exposed animals had a low or normal platelet count and severe changes in the heart and other organs. It is possible that some of the infarcts were caused by embolism. This is suggested by the frequency of cardiac vegetations in animals showing renal infarcts. Moreover, in each of the 2 rats presenting extensive cardiac infarction and septal fibrosis, respectively, there were present on the aortic valve large vegetations which could have been a source of emboli. The possibility that some of the infarcts may have been due to air embolism must also be considered, but the low incidence of infarcts in animals under 100 days suggests that this is not a major factor.

Renal infarcts and hematuria have not been reported previously as observed in animals exposed to high altitude, but hematuria has been observed in men suffering from "soroche" in the Andes¹¹. Some of the exposed rats in this study showed numerous hemoglobin casts and nearly all showed marked hemosiderosis of the kidney and less frequently of the liver, the lung and the adrenal gland. These findings may be due to excessive hemolysis, since similar changes have been noted in experimental animals following administration of hemolytic agents¹². It is noteworthy that marked renal hemosiderosis was observed in some rats with a subnormal amount of hemosiderin in the spleen.

Intestinal bleeding appeared to be one of the major contributory causes of death. Similar hemorrhages were found in rats exposed continuously to high altitude by Sundstroem and Michaels^{2a}. Gastric ulcers were not as frequent as reported in other altitude studies¹³.

Repeated daily exposure to a simulated altitude of 25,000 feet greatly reduced the life span of rats. This was probably due in many cases to infarcts, hemorrhages and other pathologic changes. The myocardial fibrosis seen in many of our rats resembled that reported

10 Harrop, G. A. *Medicine* **7** 291, 1928. Weber, F. P. *Polycythemia, Erythrosis, and Erythraemia*, London, H. K. Lewis & Company, Ltd., 1921. Addenda, London, H. K. Lewis & Company, Ltd., 1929. Weber, F. P. *Lancet* **227** 808, 1934. Boyd, W. *Am J M Sc* **187** 489, 1934. Reznikoff, P., Foot, N. C., and Bethea, J. M. *Am J M Sc* **179** 753, 1935. Cecil, R. L. *Textbook of Medicine*, Philadelphia, W. B. Saunders Company, 1944, p. 987.

11 Monge, C. *Physiol Rev* **23** 166, 1943.

12 Spicer, S. S., Highman, B., and Monaco, A. R. *J Pharm & Exper Therap* **95** 256, 1949.

13 Dalton and others^{2f}. Reynolds and Phillips^{2g}.

by Wilens and Sproul⁹ to occur spontaneously only in rats over 400 days old. We found numerous hemoglobin casts in the kidney only in control rats that were over 300 days old. In exposed animals, however, numerous casts occurred in much younger animals. These findings suggest that, in addition to producing specific lesions, hypoxia may hasten the development of lesions common in senile animals.

SUMMARY

Rats were exposed to a simulated altitude of 25,000 feet four hours daily, starting at 14 days of age. Hematocrit readings, oxyhemoglobin concentrations and erythrocyte counts increased gradually during the first 100 days and persisted at high levels. The mortality was high after 100 days of age, and none lived more than half of the normal life span.

Nearly all of the exposed rats had striking vascular engorgement and severe lesions in various organs. The heart was usually distinctly hypertrophied, the valves were often thickened, and vegetations were found principally on the mitral, and less frequently on the aortic and tricuspid valves and in the auricles. In addition, many of the rats showed fibrosis of the inner portion of the wall of the left ventricle and fatty and various other degenerative changes in the myocardium, and one rat presented coronary occlusion with infarction.

Infarcts were frequently found in the kidneys. Many kidneys also showed marked hemosiderosis and a lesser number, numerous hemoglobin casts and fatty changes in the media of some arteries or in the epithelium of some tubules. Some rats showed gross hematuria.

A large proportion of the rats that died had a massive accumulation of blood in the intestine, but only a relatively small number had ulcers in the intestine or the stomach. Hemorrhages were also found in the central nervous system.

These findings indicate that rats are unable to acclimatize fully to short daily exposures to a simulated altitude of 25,000 feet. It is suggested that discontinuous exposure of laboratory animals to simulated high altitudes may be a useful experimental method for the production and study of cardiovascular and other lesions.

EXTRAGENITAL CHORIOCARCINOMA

With Comments on the Male Origin of Trophoblastic Tissues

EDWIN F. HIRSCH, M.D.

CHICAGO

CHORIOCARCINOMA,¹ as originally defined, is a cancerous growth of the trophoblastic Langhans and syncytial cells of the placenta. The tumor, accordingly, is an atypical variant of a pregnancy—or more specifically, a cancerous growth developing from a fertilized ovum. In the fertilization of the ovum a male component is fused with a female, and the union initiates a sequential growth of tissue cells, some of which are destined to form an embryo and others to develop into a placenta. This concept of the origin of choriocarcinoma had general acceptance, without exception, until tissues resembling trophoblast were observed in cancerous teratomas, notably those of the testis. That these tumors contained trophoblastic tissues was disputed until methods were found for demonstrating gonadotropic hormones (Aschheim-Zondek reaction). Then the fluids of patients having cancerous teratoma of the testis with trophoblastic tissues were found to have a high content of these hormones.

Brewer² discussed the importance of the chorionic gonadotropin with regard to the diagnosis of testicular tumors and emphasized that the classification remained confused because two varieties of gonadotropic hormone could be present in the urine of the patients, and the significance of each one had not been evaluated properly. He quoted many articles describing the biologic properties and differentiation of these two hormones. The one hormone, the chorionic, is identical with that found in the urine of pregnant women and produces luteinization in the ovarian follicles of test animals. The other hormone, the castrate type, is elaborated by the pituitary gland and occurs in the urine of male and female castrates, in women past the menopause and in elderly men. This hormone stimulates follicle growth but not luteinization in the ovaries of test animals. Chorionic gonadotropin appearing in the urine indicates the presence of biologically active tissues in the host and is the more important in the evaluation of a testicular tumor. Brewer stated also

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1 Marchand, F. *Monatsschr. f. Geburtsh. u. Gynak.* **1**: 513, 1895.

2 Brewer, J. I. *Arch. Path.* **41**: 580, 1946.

that this hormone is produced by cells of the fetal placenta. In the early phase of pregnancy, when the chorionic gonadotropin appears in the urine, the growth of chorionic tissues greatly exceeds that of the embryo proper. As the tissues of the embryo differentiate, the titer of the urinary gonadotropin decreases. Most authors, according to Brewer, agree that this hormone reaches a peak in the urine by the sixtieth to the eighty-fourth day after the first day of the last menstrual period and then falls sharply. At about this time the Langhans cells of the chorionic tissues begin to decrease. During the remainder of the pregnancy the urinary gonadotropin is low. The tissues of the fetus, accordingly, do not produce the hormone. Supporting this conclusion is the observation that in women with abortion or tubal pregnancy the chorionic tissues survive and grow, despite the death of the embryo, and chorionic gonadotropin continues to be excreted in the urine, and also the observation that in women with choriocarcinoma the urine has high titers of chorionic gonadotropin, although no tissues of an embryo or a fetus remain. The hormone disappears from the urine of these patients when the uterus with the tumor is removed, provided no large metastases are present. Later, when these metastases develop, the hormone reappears in the urine.

Brewer stated that (1) all the evidence obtained in clinical and experimental studies indicates that the chorionic gonadotropin is elaborated by chorionic tissues, (2) the tissues of the embryo proper do not produce the hormone, (3) no other tissues are known to produce this hormone, (4) when the hormone is present in patients with testicular tumors, it is identical with the chorionic gonadotropin found in the urine of pregnant women (and those with choriocarcinoma), and (5) in the patients with testicular choriocarcinoma the hormone is produced by the trophoblastic tissues of the tumor.

Primary choriocarcinoma of the ovary is exceedingly rare. Pick³ in 1904 described a choriocancerous teratoma of an ovary of a girl aged 9 years and since then, according to a recent summary by Oliver and Horne,⁴ 12 other tumors proved to be choriocarcinoma have been reported, to which they added another, making a total of 14. Nine of the 14 tumors occurred in children below the age of 12 years, 2 more in girls 13 and 14 years of age—and pregnancy in association with an ovarian teratoma in the oldest (27 years) of the remaining 3 patients could not be excluded by the author himself.⁵ Chorionic gonadotropin tests are not recorded in the first reports of these ovarian tumors. The incidence of the ovarian choriocancerous teratomas is at an age when sex

3 Pick, L. *Klin Wchnschr* **41** 158, 1904

4 Oliver, H. M., and Horne, E. O. *New England J. Med.* **239** 14, 1948

5 Sbarcea, J. *Centralbl. f. Gynak.* **63** 936, 1939

differentiation has not been established and in females that genetically could be males

Mathieu and Robertson ⁶ stated that about 200 testicular choriocarcinomas had been reported at that time Friedman and Moore ⁷ reported 64 per cent choriocarcinomas among the embryonal carcinomas and teratocarcinomas they observed among 922 testicular tumors These embryonal carcinomas and teratocarcinomas comprised 54 per cent of the total number of testicular tumors This large number contrasts sharply with the few similar ovarian tumors reported and, of course, does not accurately reflect the actual incidence Symeonidis ⁸ and others have emphasized this overwhelming preponderance of testicular over ovarian choriocarcinomas Symeonidis stated that this fact has general biologic interest which reaches beyond the field of oncology and into that of embryology This almost exclusive development of trophoblastic tissues in the male gonad, he stated, may be due to the specific ability of the male sex cells to produce this teratoma He ventured to suggest even further that perhaps the trophoblast of the ovum develops because of influences contributed by the male component in the fertilization process The age range of the males with these tumors has interest and probably also significance They occur most often in the third and fourth decades, when full functional activity of the tissues of the testis has been established,⁷ not at the prepuberty level

Another group of choriocarcinomas (teratomas) has been described ⁹ These are extragenital tumors, and significantly all, to my knowledge, have occurred in males ranging in age from 13 to 72 years When these growths were described originally, objections were made to the diagnosis because the presence of true trophoblastic cells was disputed Later, when large amounts of chorionic gonadotropin were demonstrated in the urine of the patients, the objectors contended that these extragenital tumors were secondary to one primary in a testis not examined, or one of small, even microscopic dimension overlooked in the examination of the testis, or one destroyed and leaving only a scar tissue residue The number of these again is small Hirsch, Robbins and Houghton listed 14, to which they added another These extragenital choriocarcinomas have been described as occurring most frequently in the retroperitoneal tissues, then in the mediastinum, in the urinary bladder and, several, in the brain The origin of those observed in the retroperitoneal, mediastinal and urinary bladder tissues has been explained on the basis of germ tissue displaced from the urogenital ridge, or the equivalent, an abdominal testis

6 Mathieu, A, and Robertson, T D Internat Abst Surg 69 158, 1939

7 Friedman, N B, and Moore, R A Mil Surgeon 99 573, 1946

8 Symeonidis, A Centralbl f allg Path u path Anat 62 177, 1935

9 Hirsch, O, Robbins, S L, and Houghton, J D Am J Path 22 833, 1946

as designated by Symeonidis Staemmler¹⁰ found accessory testis tissues with germinal epithelium in the retroperitoneal fat near the origin of the inferior mesenteric artery

REPORT OF A CASE

A white man, aged 26 years, and married, entered St Luke's Hospital on July 30, 1948 because of a vague pain of four weeks' duration in the right hip which radiated to the lateral side of the knee Three weeks before, he began to have a dry, unproductive cough and an occasional pain in the right side of the chest Later the cough became productive, and the secretions were stained with

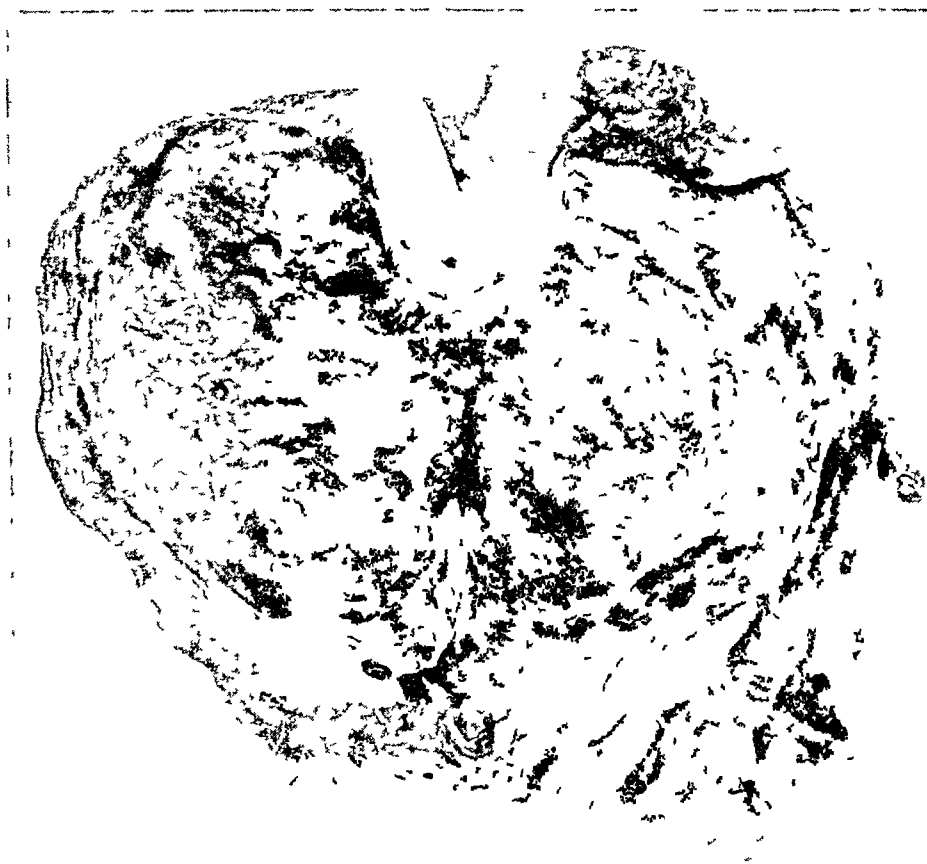


Fig 1—Surfaces made by hemisecting the extragenital (retroperitoneal) choriocarcinoma Note the close proximity of the aorta and the tumor

blood He sought medical attention and a roentgen examination of the chest disclosed changes, not specified, in the lungs A roentgenogram of the hip disclosed nothing A left orchiopexy had been done in the Army in 1943 for undescended testis The patient was well nourished He had a temperature of 99.4 F The penis was moderately undeveloped, and there was an orchiopexy scar on the left side of the scrotum The right testis, the size of an almond, was not tender or indurated, the left testis, slightly larger than the right, seemed to have a nontender nodule the size of an orange seed at the inferior pole, connected with

the epididymis. The blood had 3,900,000 erythrocytes and 10,150 leukocytes per cubic millimeter, and the hemoglobin content was 10.3 Gm per hundred centimeters. The alkaline phosphatase was 97 units. A roentgenogram of the chest demonstrated extensive metastases in both lung fields, presumably from a testicular tumor.

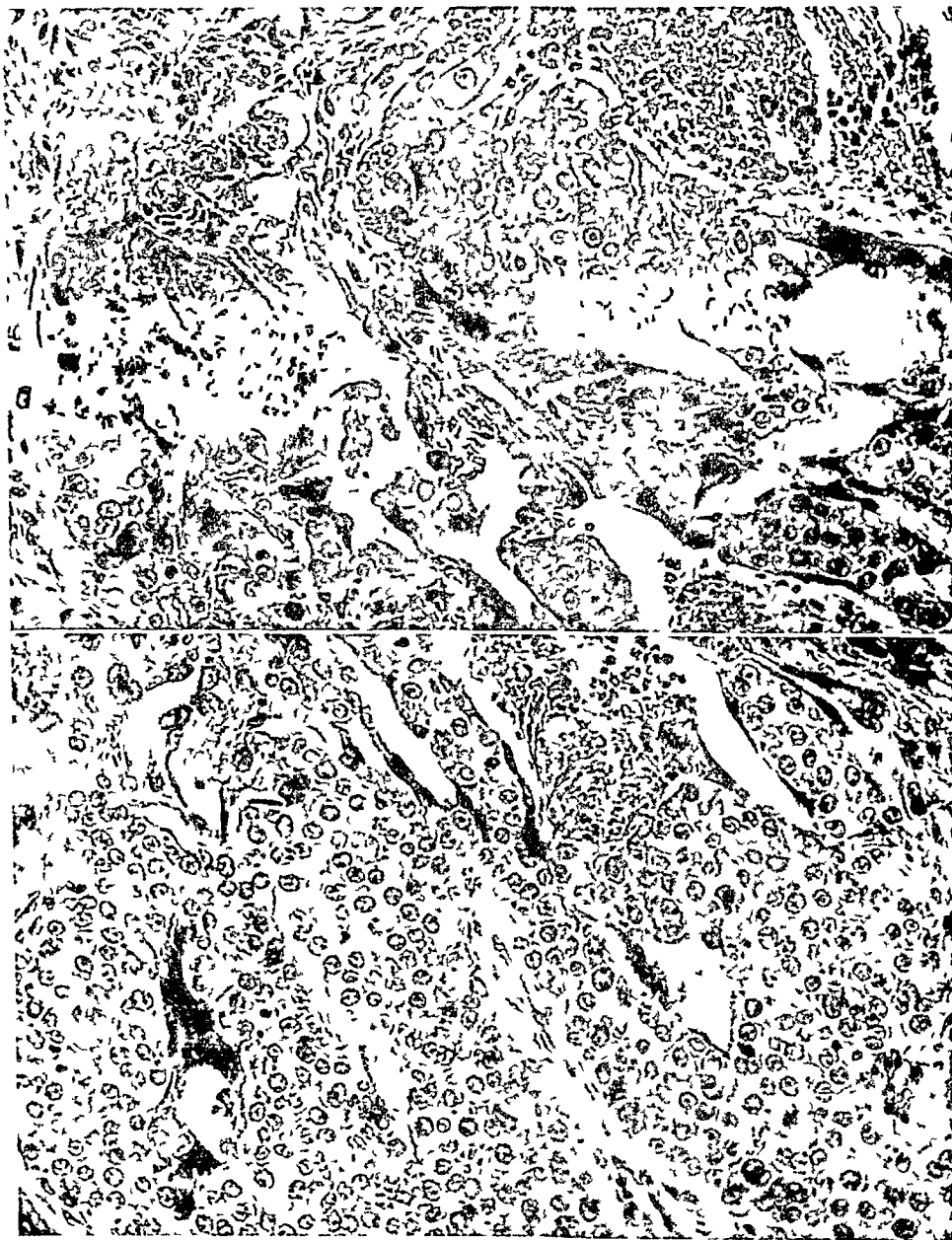


Fig 2—Photomicrographs illustrating the large syncytial and trophoblastic cells of the choriocarcinoma, $\times 198$

An Aschheim-Zondek test was negative at this time. The left testis was removed surgically on August 6, but the gross and microscopic examinations disclosed no tumor. There was distinct hypoplasia of the tubular epithelium. At this time reddish blue nodules appeared in the skin tissues of the left fifth finger, the scalp

and the face. One in the scalp, removed on August 16, was diagnosed as metastatic choriocarcinoma. Shortly after, the patient had a convulsion, considered to be on the basis of a metastasis involving the brain. Hemorrhagic metastases now appeared in the skin of the chest, trunk, arms, hands and face. His anemia became severe, and shortly before death the Aschheim-Zondek test of the urine was positive in dilutions up to 1 to 500. Death occurred on October 12. The postmortem examination of the body was limited to the trunk.

The essentials of the anatomic diagnosis are: primary choriocarcinoma of the right perinephric retroperitoneal tissues, extensive metastatic choriocarcinoma of the lungs, left axillary lymph nodes, diaphragm, stomach, right and left kidneys, verumontanum (colliculus seminalis), small bowel, cecum, scalp and subcutaneous tissues of the body, hypoplasia of the right testis, old orchidectomy scar of the left groin.

Medial to the right kidney and near the midline was a hard mass of tissue 11.5 by 5 by 4.5 cm, its upper edge behind the right renal artery (fig 1). It was firmly attached to the ligament tissues that were in front and on the right side of the spine opposite the first to fourth lumbar vertebrae. At the upper edge it had invaded the psoas muscle, but lower down the muscle tissues were displaced laterally and anteriorly. The right kidney was separate. Surfaces made by cutting the mass were mainly tan brown, hemorrhagic, leathery and fibrous tissues. No enlarged iliac lymph nodes were on either side. As indicated in the anatomic diagnosis, there were many metastases in numerous parts of the body, in the lungs especially. The body of the right testis was 4 by 2.5 by 2.7 cm. The tunics were smooth, surfaces made by cutting were brown, and threadlike tubules could be pulled out. The epididymis was small and had no tumor.

The right retroperitoneal tumor and many metastases were examined histologically. All had trophoblastic Langhans and syncytial cells, associated with extensive hemorrhages (fig 2). The right testis had atrophic or hypoplastic germinal cells without a trace of spermatogenesis. The interstitial cells were abundant and apparently increased in amount.

COMMENT

The occurrence of cancerous trophoblastic tissues in the testis and other (extragenital) male germinal tissues is accepted. Many observers discussing the origin of testicular teratomas have hypothesized a parthenogenetic or a hermaphroditic process. Fortner and Owen¹¹ apparently regarded an ovum component as an essential factor in the growth of a choriocarcinoma but doubted that the male gland possessed structures which had the morphologic value of an ovum and which, in turn, had trophoblastic tissues. Petillo,¹² in 1944, reviewed the theories concerning the pathogenesis of teratomas and choriocarcinomas of the testis. The latter, he said, should be termed embryochorionomas and not teratomas. He proposed the hypothesis that a sex reversal may occur in the human gonads which makes possible an actual fusion of ova and spermatozoa. This autofertilization initiates a growth of one or more

11 Fortner, H. C., and Owen, S. E. *Am J Cancer* **25**: 89, 1935.

12 Petillo, D. *Urol & Cutan Rev* **48**: 53, 1944.

embryos which die, leaving as survivals the chorion and occasional vestiges of other tissues. He did not state how these tissues then become cancerous instead of remaining local and limited in growth.

Petillo explained the great disparity of the testicular and the ovarian occurrence of these tumors on the basis that there is a greater opportunity for sex reversal in the testis because most oogonia have advanced to genetic maturity before puberty. Therefore, ovarian sex reversal is possible only in the first decade of life and accordingly choriocarcinoma of the ovary is a disease of childhood.

Petillo's parthenogenetic origin of teratomas of the testes proposes (1) a highly theoretic sex reversal of germinal tissues of a male which occurs long after the testes have reached functional development, (2) the maturation of this sex reversed element, a potential ovum, and (3) the fertilization of this female element by a sperm. Apparently in many theories of new growths of germinal tissues, including the choriocarcinomas, the reasoning is dominated by the idea that a process of fertilization initiates the growth. Present views of the origin of cancerous tumors of other tissues of the body do not hypothesize a union of such matured cell elements, a fertilization process, as the initiating phase of the growth. Choriocarcinoma arising in women with pregnancy is a cancerous growth of trophoblastic tissues of the placenta, a structure adventitious to the fetus but having its origin in the mass of cells resulting from fusion of an ovum and a sperm. So long as this was the only way in which these cancerous growths were known to occur, an actual fusion of the male and female elements seemed essential. Now, because choriocarcinoma is known to occur often in testes, rarely in extragenital tissues (probably germinal) in males, and in the ovaries of only a few female children or adolescents, the fertilization process as such has less significance. Trophoblastic tissues, the characteristic element of these cancerous tumors, may come from potential elements in either the sperm or the ovum, and not necessarily from the composite cells arising from both. According to this reasoning, in the origin of choriocarcinoma complicating a pregnancy, fertilization provides only some of the conditions favorable for the development of this cancerous tumor. Friedman and Moore⁷ have stated that embryonal carcinomas and teratoid tumors, which consist of evolving and differentiating somatic and trophoblastic tissues, are neoplastic expressions of embryonic cells.

In the male, choriocarcinomas arise in germinal tissues. According to Symeonidis, only teratomas arising in male germinal or pregerminal cells have the ability to produce trophoblastic tissues. Further, choriocarcinomas, whether they lie within the germ tissues or elsewhere (extragenital), he stated, are derivatives of germinal cells, so far testicular.

Accordingly, certain comments seem proper in considering the entire incidence of choriocarcinomas. In the adult female the tumor occurs in

the uterus or adnexa as a complication of pregnancy, that is, through fertilization of an ovum by which a male and a female sex element are fused. In the adult male it occurs just as exclusively in the testis or in apparent displacements of testicular tissues. The mature testis, according to the information now available concerning its structure, has no matured ova. The conclusion seems to follow that trophoblastic tissues are a characteristic of the male germinal tissues (testis), not of the mature female (ovary), and that the male element contributes the trophoblastic tissue component when choriocarcinoma develops from a fertilized ovum in the uterus or adnexa as a complication of pregnancy. The almost total absence of reports of choriocarcinoma of the ovary of the adult female supports this view. As Symeonidis has suggested, perhaps the trophoblast of the fertilized ovum forms because of influences introduced by the male component. Stated in a more direct way, the testicular choriocarcinomas seem to provide evidence that trophoblastic tissues are contributed by the male component in the normal development of a fertilized ovum in the uterus.

SUMMARY

Choriocarcinoma, originally regarded as a cancer of the trophoblastic tissues of the placenta, occurs also, in many instances, in the testis and, in a few instances, in the ovary of the young or adolescent females and, in a small number of cases, in extragenital tissues, usually retroperitoneal or mediastinal, but in these cases exclusively in the male.

Chorionic gonadotropin, the active principle of the Aschheim-Zondek reaction, appears in the urine of patients with choriocarcinoma and provides biologic evidence, in addition to the tissue structure, that the tumor contains trophoblastic elements.

Many cases of choriocarcinoma have been described among cases of cancer of the testis of the fully mature male, only a few cases of choriocarcinoma of the ovary of the young or adolescent female have been reported. In the recorded instances of extragenital choriocarcinoma the tumor was observed only in fully mature males.

The process of fertilization, parthenogenetic or hermaphroditic, has seemed convenient to use theoretically for the explanation of these tumors, probably, at first, because choriocarcinoma was recognized as a cancerous complication of pregnancy. Trophoblastic tissues theoretically may be derived from potential elements of either the sperm or the ovum, not necessarily from the composite tissues produced by the fusion of the two. Fertilization, accordingly, simply provides conditions favorable for the growth of choriocarcinoma.

Tumors of this type, exclusive of those complicating pregnancy in fertile women and a remarkably small number encountered in ovaries of

immature or adolescent females, which may be actually testicular, occur in large numbers in testes of mature males and in small numbers in extragenital tissues considered to be germinal, also in mature males

The conclusion seems to follow that trophoblastic tissues are a characteristic of mature male germinal tissues (testis), not of the mature female (ovary), and that the male element contributes the trophoblastic tissue component when choriocarcinoma develops in the uterus or adnexa as a complication of pregnancy. This could also imply that the testicular choriocarcinoma provides evidence that the trophoblastic tissues (placenta) are contributed by the male element in the normal development of a fertilized ovum in the uterus.

Another case of extragenital, retroperitoneal choriocarcinoma, in which the patient was an adult male, is recorded

ACUTE CLOSED CEREBRAL LESIONS TREATED BY INJECTION OF HYPERTONIC DEXTROSE SOLUTION AND BY SURGICAL DECOMPRESSION

A Quantitative Study

C B TAYLOR, M D

GEORGE M HASS, M D

AND

JOHN E MALONEY, M D

CHICAGO

PREVIOUS studies have shown that acute closed cerebral lesions can be produced hypothermally in rabbits without interrupting the continuity of the calvarium or introducing variables incidental to mechanical trauma¹. The lesions were controlled as to dimensions and locations so that they could be reproduced topographically and quantitatively in successive animals. Although hemorrhage, edema and necrosis varied slightly in lesions which were otherwise identical, the variations were restricted to the discrete volumes of cerebral injury. Animals rarely showed symptoms when unilateral or bilateral cerebral lesions occupied less than 94 volumes per cent of brain. Severe symptoms leading to death often developed when lesions occupied 94 to 185 volumes per cent of the brain. When lesions were within this range of magnitude, the average minimum lethal volume of cerebral damage in 50 per cent of the animals (MLV_{50}) was 143 volumes per cent of the brain. Several clinical courses with an average postoperative duration of about seven hours and fatal termination always occurred when lesions occupied more than 185 volumes per cent of the brain. The great majority of animals that died had a postoperative period of normal behavior lasting one to six hours. This was followed by a secondary lapse into stupor which was a clear indication of impending coma and eventual death, the animal succumbing within at least twenty-four hours from the time of completion of the operation.

These data indicated that the method might be useful for a study of the rate of onset and decline of factors responsible for death. Multiple

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1 Taylor, C. B., Hass, G. M., and Maloney, J. E. Arch Path **47** 450, 1949

sublethal lesions were made, successively, in animals at twenty-four, forty-eight, seventy-two and ninety-six hour intervals² These studies showed that the factors responsible for death began to subside after twenty-four hours had elapsed and by the end of forty-eight hours had largely disappeared It was suggested that cerebral congestion and edema were the most important of these factors

These observations established methods and criteria for a quantitative study of the treatment of acute closed cerebral lesions characterized by local edema, hemorrhage and necrosis It seemed that any treatment, to be effective, would have to be instituted early and that the effectiveness of the treatment could be measured, either in terms of survival of animals with lesions of lethal magnitude or in terms of survival of animals with symptoms indicating the presence of a lethal lesion Furthermore, it seemed that the degree of effectiveness of the treatment might be evaluated, quantitatively, in terms of dimensions of lesions With these points in mind a study was planned for the purpose of inquiring into the merits of intravenous injection of hypertonic dextrose solution and surgical decompression as methods of treatment

METHODS

Albino rabbits, 3 to 6 months of age and weighing 4 to 6 pounds (1,814 to 3,721 Gm), were used Closed intracerebral lesions were produced by a method described elsewhere¹ This method consisted essentially of aseptic exposure of the calvarium of the vertex of the skull followed by freezing of the calvarium and underlying cerebrum with a special instrument One or more simultaneous lesions were produced in one or both cerebral hemispheres, care being taken to produce a lethal or near lethal volume of cerebral injury The range of the quantity of damage was 89 to 512 volumes per hundred volumes of the brain The scalp was then closed with silk, and treatment was instituted at various times in the postoperative period

Twenty-four animals were treated by intravenous administration of 10 or 25 per cent aqueous solutions of dextrose (See table 1) The dextrose was given in a vein of the ear, with the head and neck of the animal immobilized by an adjustable bivalved plaster cast Under these conditions the animal ordinarily lay quietly at rest throughout the period of administration of fluid The rate of administration of dextrose solutions was controlled by a mercury drop displacement method The rate varied from as much as 50 cc per minute, when separate intravenous injections were given, to 6 to 8 cc per hour when intravenous injections were continuous

Separate injections of 10 per cent dextrose solution were given intravenously as follows Each of 6 animals was given a single injection immediately after the operation, 1 was given 17 cc and 5 received 50 cc Each of 2 animals was given a single injection at the end of the first postoperative hour, 1 was given 17 cc and 1, 50 cc Three animals received total amounts of 45, 50 and 55 cc of dextrose solution, respectively, by two or more injections at short intervals of time in the immediate postoperative period

² Tavor, C B, Hass, G M, and Maloney, J E Arch Path 48 195, 1949

Continuous administration of 25 per cent dextrose solution, 6 to 8 cc per hour, the total amounts varying from 4 to 182 cc, was used in 13 animals. The injection was continuous for twenty-four hours if the animal survived. At the end of twenty-four hours the injection was stopped, because previous experience had shown that if an animal survived for twenty-four hours it recovered completely, except for rare instances in which an animal lived for days in a lethargic, semi-stuporous state.

TABLE 1—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Intravenous Injection of Dextrose*

Percentage Volume of Cerebral Damage *	Intravenous Therapy After Production of Lesions		Duration of Life After Production of Lesions, Hr
	Method of Postoperative Intravenous Administration of Dextrose	Dextrose Concentration, Per centage Total Amount of Solution Used, Cc	
8.9	Continuous infusion	25 177	23
11.0	Continuous infusion	25 30	4
12.1	Immediate single infusion	10 50	Survived
12.6	Continuous infusion	25 178	Survived
12.8	Delayed single infusion 1½ hours postoperatively	10 50	3
13.3	Continuous infusion	25 151	Survived
14.5	Immediate single infusion	10 17	Survived
14.6	Immediate single infusion	10 50	10
14.7	Two infusions at 0 and 1 hour postoperatively	25 50	2
14.9	Continuous infusion	25 12	2.25
16.3	Two infusions at ½ and 1 hour	10 45	6
16.7	Delayed single infusion 1 hour postoperatively	10 17	3.75
16.9	Immediate single infusion	10 50	Survived
17.5	Continuous infusion	25 168	Survived
18.0	Continuous infusion	25 64	15
18.4	Continuous infusion	25 44	8
18.6	Four infusions at 0, 1, 2, 3 hours postoperatively	10 55	4
19.3	Immediate single infusion	10 50	6.5
19.5	Continuous infusion	25 182	Survived
19.6	Continuous infusion	25 20	2.75
19.7	Continuous infusion	25 12	1.25
21.2	Immediate single infusion	10 50	2.25
24.7	Continuous infusion	25 5	0.75
30.0	Continuous infusion	25 4	0.75

* The percentage volume of cerebral damage represents the number of cubic millimeters of lesion per hundred cubic millimeters of brain.

Three rabbits in which closed cerebral lesions had not been produced were given 25 per cent dextrose solution, 6 to 8 cc per hour, intravenously for twelve, twenty-four and thirty-six hours. This was done to determine the effect of this type of treatment on normal animals.

Surgical decompression was done by excision of a circular, quadrangular or elliptic piece of the calvarium. The bone was cut by a thin rotating emery disk attached to the terminal fitting of a flexible cable powered by an electric motor (a dental grinding and polishing instrument). The emery disk quickly cut through the bone at the periphery of the bone flap. The bone flap was then elevated and separated from the dura. Occasionally, a small cut was inadvertently made in the dura while the bone flap was being removed. In general, however, trauma of the brain was minor, and escape of cerebrospinal fluid was negligible.

TABLE 2—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Surgical Decompression Over Each Lesion Thirty to Forty-Five Minutes After Production of Lesion*

Dimensions of Parietal Lesions				Dimensions of Decompressions		Percentage Volume of Cerebral Damage	Duration of Life After Production of Lesions
Right		Left		Right Area, Mm ²	Left Area, Mm ²		
Surface Area, Mm ²	Depth, Mm	Surface Area, Mm ²	Depth, Mm				
154	4 0			105		9 8	Survived
243	5 0			142		10 0	Survived
228	5 0			149		11 0	Survived
149	4 5	170	5 0	61	100	12 5	Survived
351	5 0			138		12 6	Survived
290	4 5			150		13 1	Survived
325	5 0			141		13 3	Survived
355	5 0			125		13 5	Survived
342	5 5			143		14 0	Survived
327	5 0			123		14 2	Survived
385	5 0			223		14 6	16 hours
328	4 0	71	4 0	160		14 7	10 hours
387	5 0			142		14 9	Survived
390	5 0			132		15 0	Survived
330	5 0			141		15 0	7 hours
434	5 0			218		15 4	4 hours
362	5 0			118		15 6	Survived
407	5 0			142		15 7	Survived
410	5 0			181		16 4	Survived
343	6 0			198		16 5	Survived
369	6 0			154		16 6	14 hours
362	3 0	324	3 5	94	110	17 6	4 hours
54	4 5	432	4 5		109	17 8	7 hours
251	5 0	222	4 5	41	78	18 3	Survived
427	7 0			135		19 0	Survived
437	5 5			110		20 0	9 hours
312	4 0	324	4 0	88	91	20 4	5 hours
542	5 5			160		20 6	Survived
439	6 0			133		21 2	9 hours
448	6 0			120		21 3	Survived
331	5 0	314	3 0	92	100	22 4	13 hours
461	6 5			144		23 4	Survived
306	5 0	329	4 0	94	85	23 5	Survived
324	5 0	309	5 0	56	52	24 0	7 hours
294	4 0	285	6 0	65	47	24 0	Survived
333	5 0	318	4 5	105	128	26 0	18 hours
321	6 0	324	5 5	131	94	26 5	Survived
439	3 5	420	5 0	83	75	27 7	7 hours
388	5 0	360	4 5	150	95	28 5	3 hours
305	4 2	315	4 3	84	94	29 2	16 hours
416	4 0	420	4 0	80	89	29 3	3 hours
499	3 5	403	4 2	118	101	29 6	4 hours
360	4 5	358	5 0	107	99	29 9	Survived
350	5 5	350	5 5	112	118	32 3	Survived
310	7 0	250	7 0	119	125	32 7	Survived
375	6 0	393	6 0	92	101	33 5	3 hours
458	7 5	403	7 8	129	104	51 2	4 hours

because the swollen cerebral cortex herniated into the linear dural defect. The scalp was then closed with silk.

Three groups of animals were treated by surgical decompression with removal of bone flaps. Group 1 consisted of 47 animals with unilateral or bilateral lesions

(See table 2) Surgical decompression was done within thirty to forty-five minutes after production of the lesions. Bone flaps were made over the lesions. The area of bone removed over each lesion was usually one-fourth to one-half the area of the cortical surface of the lesion. (See table 2) Group 2 consisted of 4 animals with unilateral lesions. (See table 3) Surgical decompression was done within thirty to one hundred and five minutes after the production of lesions. Bone flaps were made over the cerebral hemisphere contralateral to the location of the lesion. The area of bone removed over the normal hemisphere was about one-half the area of the surface of the cortical lesion in the opposite hemisphere. Group 3 consisted of 14 animals with unilateral or bilateral lesions. Surgical

TABLE 3—*Relations Between Percentage Volume of Cerebral Damage and Survival of Animals Treated by Surgical Decompression*

Percentage Volume of Cerebral Damage	Hours Between Production of Lesions and Onset of Symptoms		Hours Between Production of Lesions and Surgical Decompression		Duration of Life After Production of Lesions, Hr
	Stupor	Convulsions	Over Lesions	Contralateral to Lesions	
11.7				1.50	Survived
12.6				0.50	4.00
13.5	5.0			1.75	5.25
15.2	5.0			1.50	5.50
12.3		1.5			1.50
12.4					Survived
14.8		1.5			1.50
17.0	2.0	2.75			2.75
19.1		1.00			1.00
19.2	4.0		4.0		Survived
19.4		3.50	3.50		Survived
19.6	4.0		4.00		Survived
20.5		1.00			1.00
21.0	1.5		1.50		1.75
21.5	6.0		6.00		Survived
22.2		1.00	1.00		Survived
24.7	1.5		1.75		Survived
49.4		0.75	1.00		2.25

decompression was delayed until the postoperative onset of stupor or convulsions, symptoms regularly indicative of a fatal prognosis in untreated animals. These symptoms developed within one to six hours after the production of lesions. In this group the decompression had to be done rapidly, because in untreated animals death often promptly followed the onset of symptoms. It was possible to do a decompression in only 8 of the 14 animals. Five died before the operation could be undertaken. One survived without symptoms. (See table 3) Decompressions were done over the lesions. Each flap of bone removed had a surface area equal to about one-fourth to one-half the surface area of the subjacent cortical lesion.

Postmortem studies were made in all instances. Animals which survived were killed at the end of twenty-four hours. The volume per cent of cerebral damage was determined by methods described elsewhere.¹ In rabbits it represents the number of cubic millimeters of lesion per hundred cubic millimeters of brain. By study of these three groups of animals, we were able to compare, first, the relative merits of homolateral and contralateral decompression and, second, the relative merits of immediate and delayed homolateral decompression.

RESULTS

Six animals were given a single intravenous injection of a 10 per cent aqueous solution of dextrose immediately after the production of cerebral lesions (See table 1) Three lived and 3 died In those that lived the cerebral damage totaled 121, 145 and 169 volumes per hundred volumes of the brain In those that died the cerebral damage totaled 146, 193 and 212 volumes per cent of the brain The MLV_{50} was 145 volumes per cent This was comparable with 143 volumes per cent in a previously reported untreated control series¹ The maximum

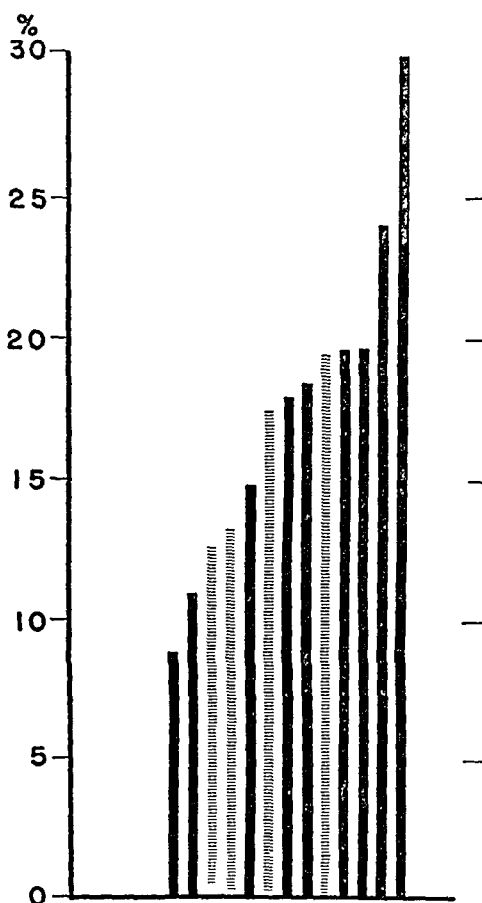


Chart 1—Each bar represents a rabbit given an intravenous injection of a 25 per cent aqueous solution of dextrose, continuously, after production of the percentage volume of cerebral damage indicated by the height of the bar The interrupted bars represent animals which survived and the solid bars animals which died

quantity of survivable cerebral damage was 169 volumes per cent This was comparable with 180 volumes per cent in a previously reported control series¹ It seemed that treatment of this type had little or no promise, so the experiment was terminated.

Two animals were given a single intravenous injection of a 10 per cent aqueous solution of dextrose a short time after the production of cerebral lesions (See table 1) They died with cerebral damage of 128 and 168 volumes per cent,

respectively. As indicated by data discussed in the preceding paragraph, this method showed little promise of benefit and was dispensed with.

Three animals were given a combination of immediate and delayed postoperative intravenous injections of 10 per cent dextrose (See table 1). All died with cerebral damage of 147, 163 and 186 volumes per cent, respectively. As indicated before, the results showed that the treatment had little promise, and it was not studied further.

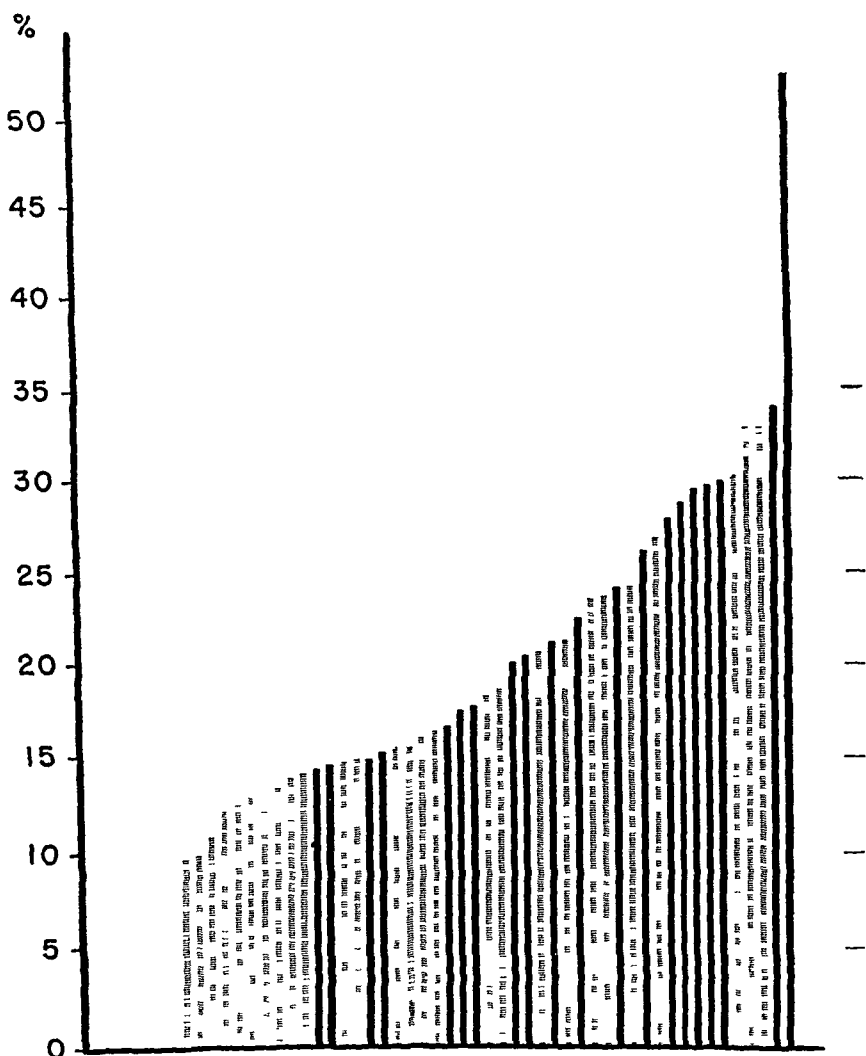


Chart 2—Each bar represents a rabbit whose brain was surgically decompressed within thirty to forty-five minutes after production of the percentage volume of cerebral damage indicated by the height of the bar. The interrupted bars represent animals which lived and the solid bars animals which died.

Thirteen animals were given continuous intravenous injections of 25 per cent dextrose (See table 1). The administration was started immediately after the production of cerebral lesions and continued at the rate of 6 to 8 cc per hour until the animal died or until complete recovery was apparent at the end of twenty-four hours. Nine animals died. Four survived. Those which died had cerebral damage of 89, 110, 180, 184, 196, 197, 247 and 300 volumes per cent, respectively. In those which survived the cerebral damage totaled 126, 133, 175

and 19.5 volumes per cent, respectively. Calculations indicated that the MLV_{∞} was about 14.5 volumes per cent of the brain (See chart 1). The maximum survivable quantity of cerebral damage was 19.5 volumes per cent of the brain. Comparison of these data with those given previously for the control series of untreated animals showed that this treatment was of no significant value. It seemed possible that too much dextrose might have been given in some instances, but similar treatment of normal animals, not operated on, had no adverse effect. Hence, further plans to seek an effective means of getting a beneficial result with hypertonic solution of dextrose were given up.

Surgical decompression with the bone flap being lifted on the side opposite the location of the cerebral lesion was done in 4 animals (See table 3). Three died with cerebral damage of 12.6, 13.5 and 15.2 volumes per cent, respectively. One lived with cerebral damage of 11.7 volumes per cent. These data indicated that the operation was of no benefit and seemed to decrease the chance of survival. Hence, no further operations of this type were done.

Surgical decompression with the bone flap being made over each lesion within thirty to forty-five minutes after production of the lesions was done in 47 animals (See table 2). Some animals had unilateral and some bilateral bone flaps. As a rule, the area of decompression was one-fourth to one-half the surface area of the subjacent cortical lesion. Smaller decompressions were relatively ineffective and were dispensed with in early experiments. Larger decompressions were difficult to do, technically. The quantity of cerebral damage varied from 9.8 to 51.2 volumes per cent of the brain. All animals with less than 14.6 volumes per cent survived. This value was considerably greater than the 9.4 volumes per cent of a previously reported untreated control series¹. The maximum survival quantity of cerebral damage was 32.7 volumes per cent of the brain. This was also much greater than the 18.0 volumes per cent in a previously reported untreated series. There were 35 animals with a quantity of cerebral damage in the range 14.6 to 32.7 volumes per cent of the brain, 17 survived and 18 died. The MLV_{∞} was about 21.9 volumes per cent of the brain (See chart 2). This was about 50 per cent greater than the MLV_{∞} (14.3 volumes per cent) of the previously reported untreated control series¹.

Surgical decompression was delayed in 8 animals until the onset of stupor or convulsions (See table 3). These symptoms, indicative of a fatal lesion in untreated rabbits, developed within forty-five minutes to six hours after production of lesions. Decompressions were then done by removing bone flaps over the lesions. Six animals survived with cerebral damage of 19.2, 19.4, 19.6, 21.5, 22.2 and 24.7 volumes per cent, respectively. One animal died with 21.0 volumes per cent and another with 49.4 volumes per cent. The average quantity of cerebral damage among the 6 surviving animals was 21.1 volumes per cent of the brain. This beneficial result was of about the same magnitude as that achieved by immediate decompression over the lesions.

COMMENT

Previous studies have shown that discrete cerebral lesions characterized by acute necrosis, hemorrhage and edema can be reproduced, topographically and quantitatively, in successive animals without opening the skull or employing methods which subject the brain to mechanical trauma¹. It was shown that in rabbits the average minimum lethal quantity of cerebral damage was 14.3 per cent of the brain by volume

No animal survived with a quantity of damage in excess of 180 per cent. It was observed that most animals which died had a period of normal postoperative behavior. Usually, this lasted one to six hours and was followed by stupor and coma, terminated by convulsions and death within twenty-four hours after the production of lesions. Further studies disclosed that the factors responsible for death continued to exert a progressive effect, if the animal survived, until about twenty-four hours postoperatively.² After this time the influence of the factors subsided rapidly, so that at the end of forty-eight hours it was possible to produce a second acute lesion of nearly lethal dimensions with survival of the animal. The evidence indicated that cerebral congestion and edema in and around the lesion were the most important factors.

There were reasons for believing that the experimental situation created by these lesions was a fair anatomic reduplication of the situation which exists following some vascular accidents occurring in the human cerebrum or in intracerebral tumors. In patients the acute onset of symptoms and the frequent rapid progression of severe symptoms lead often to a critical condition which may be treated in a variety of ways. If the condition is secondary to an intracerebral vascular accident, usually in an elderly person, either nothing is done or some form of dehydration therapy, such as the intravenous injection of a hypertonic solution of dextrose, is employed. If the condition is secondary to trauma of the head, dehydration therapy with a course of watchful waiting is the usual method of treatment. There is little sound experimental basis for divergence of views as to a proper method of treatment in any instance. The prevailing methods have largely been developed through experience. The difficulty lies in comparing, quantitatively and topographically, the lesions in one case with those in another. Views which are clearly defended by adequate data are difficult to find.

For these reasons the present experiments were undertaken. The results showed that in dealing quantitatively with reproducible acute closed cerebral lesions the intravenous injection of a hypertonic solution of dextrose, whether continuous or intermittent, was of no benefit to the animal from the point of view of either amelioration of symptoms or survival. These data do not deny the utility of dextrose as a source of nutrition. They simply cast doubt on the utility of intravenous injections of hypertonic solutions of dextrose as a means of combating the intracranial factors which lead to severe cerebral symptoms and death from cerebral causes.

On the other hand, the data showed that surgical decompression was of benefit if certain rules were followed in doing the decompression. First decompression was of little or no benefit unless it was done over the site of the lesion. Second, the area of bone removed had to be at least one-fourth to one-half the submeningeal surface area of the lesion.

to obtain a satisfactory benefit Third, although decompression done shortly after the production of lesions was more beneficial than delayed decompression, the difference was not significant When the rules suggested were followed, severe cerebral symptoms were ameliorated in many animals and the average amount of cerebral damage which could be tolerated with survival was increased about 50 per cent (See chart 3)

In view of these findings it may be worth while to reevaluate surgical decompression as a method of treating selected patients for intracerebral vascular accidents The prospective use of this method of treatment should be guided largely by the ability of the clinician to make an

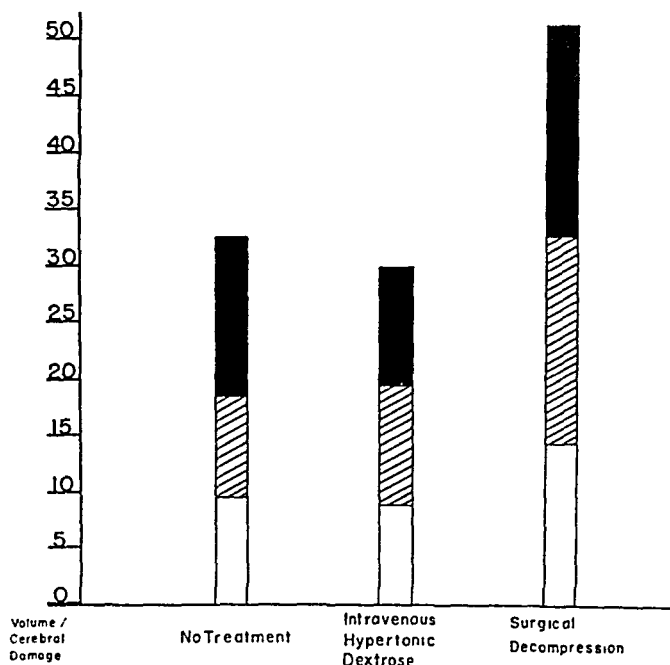


Chart 3—This graph compares experience gained in a study of three groups of rabbits with acute cerebral damage One group, previously reported, was a large untreated series¹ The group treated by intravenous injection of dextrose solution and that by surgical decompression over the sites of the cerebral lesions as described in this paper are represented in the second and third bars In these bars the lethal range of cerebral damage is shown by solid black (no survival), the survivable range by stripping (41, 63 and 51 per cent mortality, respectively), the survivable range, by solid white (no mortality)

accurate anatomicopathologic diagnosis and prognosis before considering decompression as a life-saving measure

SUMMARY

Acute closed cerebral lesions characterized by hemorrhage, necrosis and edema were produced in rabbits with a hypothermal instrument placed against the external table of the calvarium Following the pro-

duction of lesions, some animals were treated by intravenous injection of either 10 or 25 per cent aqueous solutions of dextrose and some by surgical decompression. There was no evidence that hypertonic solutions of dextrose given periodically or continuously by the intravenous route had any influence on the clinical course or the mortality rate. There was good evidence that surgical decompression was of great benefit if certain rules were followed. Removing a bone flap on the side opposite the site of the lesion was of no benefit. Removing a bone flap over the site of the lesion was of definite benefit, so that it was possible to save lives of animals, even after onset of symptoms indicating impending death, with a volume of cerebral damage averaging 50 per cent more than the average volume tolerated without treatment. The data indicated that surgical decompression should be reevaluated as a method of treatment for patients with certain types of intracerebral vascular accidents.

EFFECTS OF PROLONGED ADMINISTRATION OF ANTITHYROID COMPOUNDS ON THE THYROID AND OTHER ENDOCRINE ORGANS OF THE RAT

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THE IMMEDIATE effects of antithyroid drugs are well known, but the changes produced in the thyroid gland and other tissues by prolonged administration of these compounds have not been adequately described

Rats fed a rape seed diet for ten months or longer revealed benign adenomas in the thyroid gland,¹ while rats given 0.25 per cent thiourea in their drinking water for twenty months or longer showed carcinomas.² Female mongrel dogs receiving thiouracil for six months from the time of weaning had undeveloped gonads and accessory sex organs, abnormal dentition, and delayed growth and epiphyseal closure of the long bones³, after a detailed study of the teeth and skulls of these dogs, English⁴ presented evidence of adaptation to the effects of thiouracil. Cats responded atypically with atrophy of the thyroid gland, changes in the male gonads and necrosis of the adrenal cortex.⁵

During a study of the effects of prolonged administration of antithyroid compounds in rats, we encountered changes in the thyroid gland and the anterior lobe of the pituitary gland not heretofore reported. We also observed adenoma and carcinoma in the anterior lobe of the pituitary gland, the adrenal glands and elsewhere.

MATERIALS AND METHODS

The antithyroid compounds were administered for fourteen weeks to two years in the standard laboratory diet (Purina® dog chow) of white rats from weaning

From the Wyeth Institute of Applied Biochemistry, Wyeth Incorporated, and the University of Pennsylvania, Graduate School of Medicine

1 Griesbach, W. E., Kennedy, T. H., and Purves, H. D. *Brit J Exper Path* **26** 18, 1945

2 Purves, H. D., and Griesbach, W. E. *Brit J Exper Path*. **28** 46, 1947

3 Seifter, J. *Federation Proc* **6** 370, 1947

4 English, J. A. *J Dent Research* **28** 172, 1949

5 McClosky, W. T., Lilhe, R. D., and Smith, M. I. *J Pharmacol & Exper Therap* **89** 125, 1947

until death. Control groups received unadulterated standard diet. The animal quarters were air conditioned and maintained at 72 F.

The fate and the disposition of all the rats in the present study were as follows. Four rats received 0.05 per cent para-aminobenzoic acid continuously in the diet, 1 of these was killed at the end of fourteen weeks and 3 at the end of thirty-five weeks. Four rats received 0.1 per cent para-aminobenzoic acid continuously in the diet, 1 was killed at fourteen weeks, 1 died spontaneously after twenty weeks and 2 were killed at the end of thirty-five weeks. Five rats received 0.5 per cent para-aminobenzoic acid continuously in the diet, 1 was killed at the end of twelve weeks, 1 at twenty-four weeks and 3 at fifty weeks.

Four rats received 0.001 per cent thiouracil continuously in the diet, 1 was killed at the end of fourteen weeks, and 3 were killed at thirty-five weeks. Twelve rats received 0.005 per cent thiouracil in the diet, 5 died spontaneously, 1 at five weeks, 1 at thirty-five weeks, 1 at fifty-eight weeks, 1 at seventy-six weeks and 1 at one hundred and three weeks, 7 were killed, 1 at five weeks, 1 at fourteen weeks, 2 at thirty-five weeks, 2 at fifty-two weeks and 1 at one hundred and four weeks. Eight rats received 0.01 per cent thiouracil in the diet, 4 died spontaneously, 1 at eighty-seven weeks, 1 at eighty-nine weeks and 2 at ninety-two weeks, 4 were killed, 2 at fifty-two weeks, 1 at eighty weeks and 1 at one hundred and four weeks.

Four rats received 0.001 per cent thiouracil plus 0.05 per cent para-aminobenzoic acid continuously in the diet, 1 was killed at the end of fourteen weeks, and 3 were killed at the end of thirty-five weeks. Four rats received 0.005 per cent thiouracil plus 0.1 per cent para-aminobenzoic acid, 1 was killed at the end of fourteen weeks, and 3 were killed at the end of thirty-five weeks. The acid was included in order to boost the goitrogenic effect without increasing toxicity. Antithyroid action,⁶ low toxicity⁷ and detoxifying effect⁸ for certain metallo-organic compounds have been reported for para-aminobenzoic acid.

Four rats received 0.001 per cent 2-aminothiazole continuously in the diet, 3 were killed, 1 at the end of fourteen weeks and 2 at thirty-five weeks, 1 was missing at the end of twenty-five weeks. Four rats received 0.005 per cent 2-aminothiazole in the diet, 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent 2-aminothiazole plus 0.05 per cent para-aminobenzoic acid continuously in the diet, 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole plus 0.1 per cent para-aminobenzoic acid in the diet, 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Five rats were fed 0.1 per cent 2-aminothiazole plus 0.5 per cent para-aminobenzoic acid in the diet, 1 died spontaneously at the end of six weeks, and 4 were killed, 1 at twelve weeks and 3 at fifty weeks.

Four rats received 0.001 per cent 2-aminothiazole hydrochloride continuously in the diet, 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole hydrochloride, 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent 2-aminothiazole hydrochloride plus 0.05 per cent para-aminobenzoic acid continuously in the diet, 1 died spontaneously at the end of five weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks. Four rats received 0.005 per cent 2-aminothiazole hydrochloride plus 0.1 per cent para-aminobenzoic acid in the diet, 1 died spontaneously at the end of twenty-five weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks.

6 Berman, L. *Proc Soc Exper Biol & Med* **59** 70, 1945.

7 Richards, R. K. *Federation Proc* **1** 71, 1942.

8 Sandground, J. H. *Science* **97** 74, 1943.

Four rats received 0.001 per cent acetyl-2-aminothiazole continuously in the diet, 1 died spontaneously at the end of thirty-four weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks. Four rats received 0.005 per cent acetyl-2-aminothiazole in the diet, 4 were killed, 1 at fourteen weeks and 3 at thirty-five weeks.

Four rats received 0.001 per cent acetyl-2-aminothiazole plus 0.05 per cent para-aminobenzoic acid continuously in the diet, 4 were killed, 1 at the end of fourteen weeks and 3 at thirty-five weeks. Four rats received 0.005 per cent acetyl-2-aminothiazole plus 0.01 per cent para-aminobenzoic acid in the diet, 1 died spontaneously at the end of thirty weeks, and 3 were killed, 1 at fourteen weeks and 2 at thirty-five weeks.

Eight rats received 0.0005 per cent bis-(4-acetaminophenyl) selenium dihydroxide continuously in the diet, 5 died spontaneously, 1 at seventy-five weeks, 2 at seventy-six weeks, 1 at eighty-six weeks and 1 at eighty-eight weeks, 3 were killed, 2 at fifty-two weeks and 1 at one hundred and four weeks. Eight rats received 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide in the diet, 4 died spontaneously, 2 at sixty-six weeks, 1 at sixty-eight weeks and 1 at seventy-six weeks, 4 were killed, 1 at twenty-five weeks, 1 at twenty-seven weeks, and 2 at fifty-two weeks. Eight rats received 0.001 per cent bis-(4-acetaminophenyl) selenium dihydroxide in the diet, 6 died spontaneously, 1 at twenty-six weeks, 1 at fifty-six weeks, 1 at fifty-seven weeks, 1 at fifty-eight weeks, 1 at seventy-five weeks and 1 at ninety-five weeks, 2 were killed at the end of fifty-two weeks.

RESULTS

The amounts of antithyroid compounds administered did not materially affect the survival rate if the administration was not extended beyond thirty-five weeks. The mortality rate of rats receiving thiouracil and bis-(4-acetaminophenyl) selenium dihydroxide for one year or more was elevated 50 and 63 per cent, respectively, over the rate of 37.5 per cent of rats on the standard diet. Postmortem studies of 21 dead rats revealed pneumonia in 14, renal disease in 2 and carcinoma in 2. No cause of death was found in 3 rats.

The table lists the weights of the thyroid gland, the pituitary gland, the adrenal glands, and the thymus of each rat killed after one to two years of treatment. The thyroid glands of our untreated rats weighed less than those recorded by Donaldson.⁹ The ratio of thyroid weight to body weight in the 50 Gm rats of our series was 0.13 mg per gram of body weight,¹⁰ whereas in the Donaldson series it was 0.22 mg per gram. If this difference is taken into consideration, it can be calculated from Donaldson's figures that in our animals the normal ratio should have been 0.11 mg per gram for rats weighing 100 Gm and 0.09 mg per gram for those weighing 200 and 300 Gm. The table also shows that the thyroid glands of the 2 year old controls had the expected weight. One of the glands of the 1 year old controls was smaller than expected, and the other was considerably enlarged. The latter change was due to the fact that the gland was infiltrated with lymph follicles, presenting a microscopic picture resembling struma lymphomatosa of man (fig 1B).

Thyroid Glands—The thyroid glands of the rats receiving thiouracil were about three times the normal size as compared with the controls. The increase

9 Donaldson, H. H. *The Rat*, Bulletin no. 6, Philadelphia, Wistar Institute of Anatomy and Biology, 1915.

10 Seifter, J., and Ehrich, W. E. *J. Pharmacol. & Exper. Therap.* 92: 303, 1948.

appears to be related to the concentration of thiouracil in the diet, for it was somewhat greater with 0.01 per cent than with 0.005 per cent. The only thyroid gland that failed to enlarge to this extent was in rat 16, which had a carcinoma of the anterior lobe of the pituitary gland. In a group of 4 rats weighing 50 Gm the thyroid gland was only twice the normal size after ten days' feeding of 0.01 per cent thiouracil in the diet.

The thyroid gland was enlarged 22 to 43 per cent in 5 of 7 rats receiving bis-(4-acetaminophenyl) selenium dihydroxide. Of the 2 with a normal size gland, rat 11 had a very small pituitary gland and rat 7 received the smallest dose of the selenium compound administered.

TABLE 1—Weights of Tissues of Rats Receiving Antithyroid Compounds for One and Two Years

	Sex	No	Body Weight, Gm	Weight of							
				Thyroid Gland		Pituitary Gland		Adrenal Glands		Thymus	
				Mg	Mg per Gm	Mg	Mg per Gm	Mg	Mg per Gm	Mg	Mg per Gm
One Year											
Control	M	1	320	14.9	0.047	12.0	0.0375	33.8	0.106	117.2	0.366
	F	2	184	29.6	0.161	11.3	0.061	74.9	0.407	252.2	1.37
Thiouracil 0.005%	M	3	210	57.7	0.275	18.5	0.008	33.1	0.158	171.9	0.819
	F	4	189	45.8	0.242	16.0	0.085	60.4	0.320	196.4	1.04
0.01%	M	5	283	69.8	0.247	9.1	0.032	26.9	0.095	125.6	0.444
	F	6	172	54.0	0.314	15.0	0.087	51.7	0.301	132.0	0.767
Bis (4 acetaminophenyl) selenium dihydroxide 0.0005%	M	7	268	24.3	0.091	17.6	0.066	37.3	0.139	183.5	0.685
	F	8	150	21.4	0.143	10.6	0.071	37.2	0.248	175.7	1.17
0.001%	M	9	294	35.1	0.119	21.1	0.072	35.2	0.119	132.0	0.449
	F	10	173	24.5	0.142	16.9	0.098	65.2	0.377	153.5	0.888
	M	11	255	19.3	0.076	9.6	0.038	50.8	0.199	110.2	0.432
	F	12	165	20.1	0.122	8.4	0.051	63.6	0.385	110.0	0.667
Two Years											
Control	M	13	339	38.2	0.113	12.5	0.037	34.6	0.102	27.5	0.081
	M	14	348	35.5	0.102	10.5	0.030	36.3	0.104	66.5	0.191
	F	15	220	18.8	0.085	24.6	0.112	93.4	0.425	100.0	0.455
Thiouracil 0.005% 0.01%	M	16	271	42.0	0.155	85.5	0.315	58.5	0.216	86.0	0.317
	M	17	254	70.1	0.276	11.2	0.044	34.2	0.135	139.1	0.548
Selenium 0.0005%	F	18	217	24.1	0.111	82.5	0.150	125.2	0.577	101.0	0.465

All the thiouracil-treated rats showed on microscopic examination diffuse goiter involving the entire gland. Most of the follicles of rats 3, 4, 5 and 6, treated for one year, were not only considerably smaller than in the acute phase of hyperplasia but actually slightly smaller than normal (fig 1C). The epithelial lining was tall, and the lumen contained little or no colloid. The degree of hyperplasia resembled that found in acute experiments with 0.01 per cent thiouracil. It was 3 plus, according to our previous grading¹⁰. Although most of the follicles were hyperplastic, there were single or small groups of considerably distended follicles filled with thick colloid and lined with very flat epithelium. The latter were scattered throughout the hyperplastic glands (fig 1C). The thyroid gland of rat 3 also contained a few adenomas with fairly tall and deeply staining epithelium.

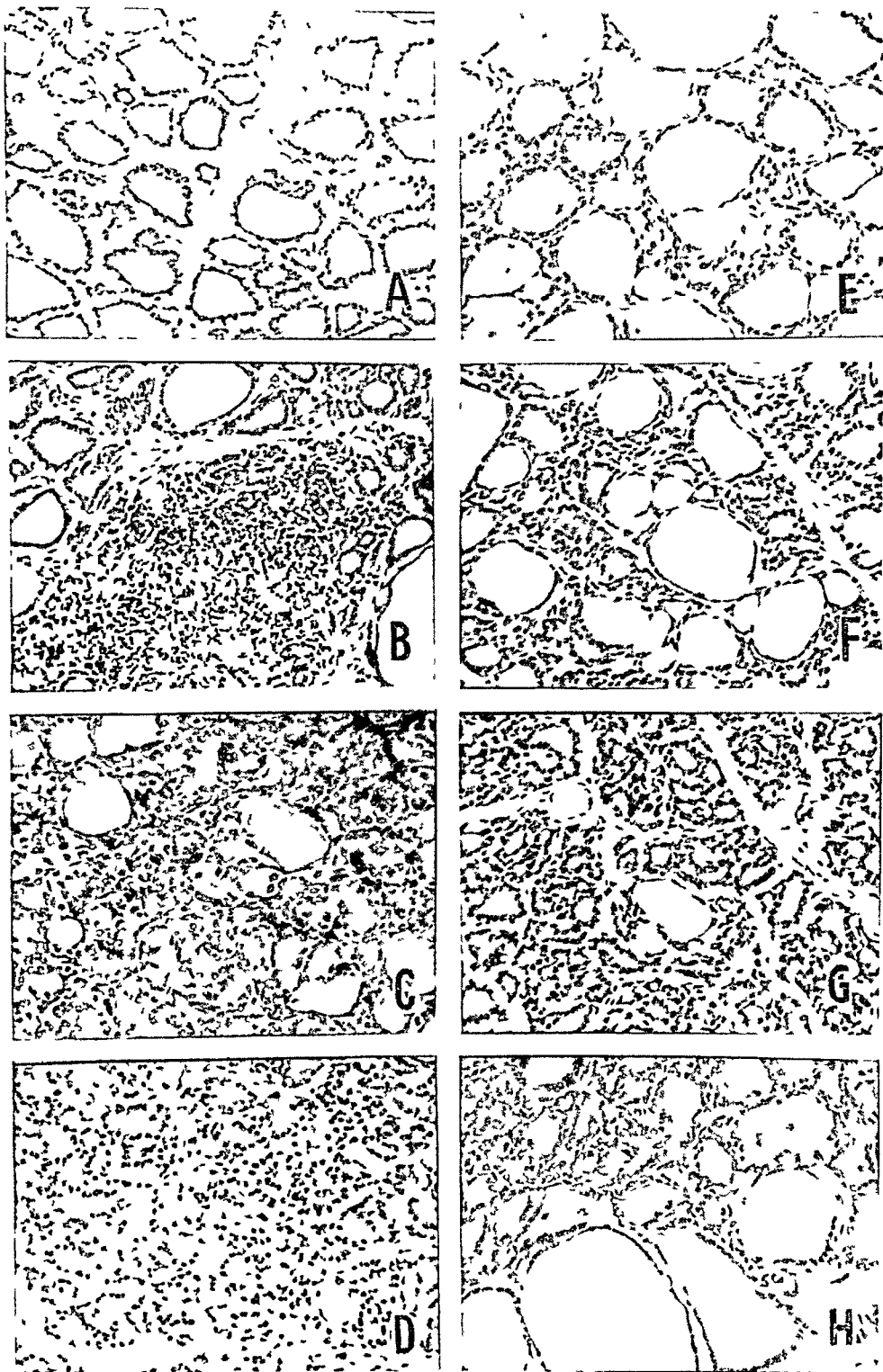


Fig 1—All the sections were stained with hematoxylin and eosin. The magnification was $\times 200$. *A*, normal thyroid gland of rat 22, an untreated control. *B*, struma lymphomatosa of rat 2, an untreated control. *C*, the hyperplasia is in the diffuse hyperplasia of thyroid gland of rat 3, fed thiouracil, 0.005 per cent, for one year, transitional stage, with some colloid-containing follicles. *D*, diffuse hyperplasia microfollicular stage, in rat 17, fed thiouracil, 0.01 per cent, for two years. Note scarcity of colloid-containing follicles. *E*, macrofollicular hyperplasia (+) in rat 23, fed thiouracil, 0.001 per cent, for fourteen weeks. *F*, microfollicular hyperplasia with many colloid-containing follicles in rat 24, fed thiouracil, 0.001 per cent, for thirty-five weeks. *G*, diffuse microfollicular hyperplasia in rat 25, fed thiouracil, 0.005 per cent, for thirty-five weeks. Note scarcity of colloid-containing follicles. *H*, focal hyperplasia with preservation of peripheral follicles in rat 12, fed a selenium compound, 0.005 per cent, for one year.

The follicles of the rats killed after two years of thiouracil administration (rats 16 and 17) were minute and lined with large epithelial cells and contained little or no colloid (fig 1D). These thyroid glands had no adenomas and only few distended colloid-containing follicles. They resembled the thyroid glands seen in microfollicular goiters in man, described by Smith and Gault¹¹

Administration of thiouracil for periods of less than one year also resulted in interesting changes (rats not listed in the table). The thyroid gland of a rat examined after 14 weeks of administration of 0.001 per cent thiouracil had slight hyperplasia of the usual macrofollicular type (+) (fig 1E). A mixture of minute, slightly hyperplastic follicles of the microfollicular type and large colloid-containing follicles with very low epithelium appeared after thirty-five weeks of thiouracil treatment (fig 1F). Increasing the concentration of thiouracil to 0.005 per cent resulted after fourteen weeks in diffuse macrofollicular hyperplasia of the thyroid gland (+++), including some distended follicles filled with colloid and lined with very low epithelium. Diffuse microfollicular goiter appeared after thirty-five weeks of this concentration (fig 1G). The addition of para-aminobenzoic acid to the thiouracil resulted in slightly greater hyperplasia. There were no adenomas in the rats receiving thiouracil for less than one year.

The selenium-treated rats had only focal hyperplasia (+ to ++), limited to the interior of the glands, while the peripheral follicles were enlarged and filled with colloid (fig 1H). There were no distended colloid-containing follicles in the interior of the glands. There were no adenomas. The follicles in the interior of the thyroid gland of rat 18, killed after two years' treatment with the selenium compound, had the identical microfollicular structure seen in the thiouracil-treated rats. In addition, however, the gland contained a solid adenoma consisting of apparently atypical thyroid epithelial cells (fig 2A) with questionable mitotic figures.¹²

The thyroid glands of rats receiving 0.001 per cent of 2-aminothiazole or its derivatives for fourteen or thirty-five weeks had little or no focal hyperplasia, while all those receiving 0.005 per cent had some focal hyperplasia. Co-administration of para-aminobenzoic acid resulted in somewhat greater hyperplasia. Of 4 rats receiving 0.1 per cent 2-aminothiazole together with para-aminobenzoic acid, the one killed after fourteen weeks showed marked diffuse hyperplasia of the usual type (+++) but slightly greater than that observed in rats receiving only 2-aminothiazole for eleven days.¹⁰ Of the 3 remaining rats killed after one year of administration of 2-aminothiazole and para-aminobenzoic acid, 1 showed a typical microfollicular goiter, in the other 2 the thyroid gland had well advanced changes indicative of transition to this stage. One of these (rat 19) also contained an adenoma.

Administration of 0.05 or 0.1 per cent para-aminobenzoic acid caused little or no focal hyperplasia, but 0.5 per cent produced some focal hyperplasia in all animals studied.

Pituitary Glands—The pituitary glands of rats killed after one year's administration of antithyroid compounds were considerably enlarged except those of

11 Smith, L. W., and Gault, E. S. *Essentials of Pathology*, D. Appleton-Century Company, Inc., 1938.

12 Two rats not included in this study, which died after receiving 0.075 per cent of the selenium compound for seven months, had extensive thyroid adenomas (fig 2B). The mother tissue was diffusely and definitely hyperplastic, although follicles were smaller than during the acute phase of hyperplasia.

the 2 rats receiving the large dose of the selenium compound and rat 5, receiving 0.01 per cent thiouracil. The latter contained the largest thyroid gland and the smallest adrenal glands of the lot. The unenlarged pituitary glands had no spectacular microscopic changes, but 3 of the 5 enlarged glands showed a considerable increase in "degranulated" cells, i.e., in immature granulated cells or chromophobes, thyroidectomy cells were not present.

Greatly enlarged pituitary glands which showed circumscribed tumors consisting of chromophobe cells surrounded by partially compressed essentially normal tissue were seen in 2 of 3 rats killed after two years. One of these received thiouracil and the other the selenium compound. The tumor of the selenium-treated rat contained a considerable amount of iron pigment and consisted of well differentiated chromophobes showing no mitotic activity (fig 2C), while the tumor of the thiouracil-treated rat revealed large atypical chromophobes with large, partly giant nuclei showing hyperchromasia and large orange-stained nucleoli, as well as abundant cytoplasm including a large Golgi apparatus (figs 2D and E). Abundant mitotic figures were also found. Invasion was not demonstrable, but the cytologic picture could be interpreted only as indicating cancer¹³. The third rat (17) also had received 0.01 per cent thiouracil but showed no enlargement of the anterior lobe of the pituitary gland, it also contained the largest thyroid and smallest adrenal glands of the lot.

Adrenal Glands—The adrenal glands of the thiouracil-treated rats were usually smaller than those of the controls, those of the selenium-treated rats were essentially unchanged. Enlargement of the glands was encountered in 3 rats (8, 16 and 18). That of the glands of 16 and 18 is accounted for by marked cystic degeneration of the cortex and that of rat 11 by multiple cortical adenoma. The small size of the adrenal glands of rat 8 is possibly explained by the fact that this animal was suffering from an infectious disease and that it weighed least of the lot.

The only unusual finding in the adrenal glands was that in rat 17, which had received 0.01 per cent thiouracil for two years. Both cortices had discrete tumors consisting of immature medullary cells with large nucleoli and numerous mitotic figures. The tumor cells were infiltrating the cortical tissue (fig 2F). These observations suggest that these tumors were not benign adenomas but medullary carcinomas (pheochromocytomas).

Cortical adenomas were found also in 2 rats not listed in the table. One (rat 20) died in the one hundred and fourth week of receiving 0.005 per cent thiouracil, and the other (rat 21) was killed in the thirty-sixth week of receiving 2-aminothiazole. The adenoma of the latter consisted of reticularis and had mitotic figures.

Other Tissues—The gonads of the animals did not reveal significant changes.

The thymuses of the rats receiving the highest concentrations of thiouracil or selenium for one year were smaller than those of the controls but were larger than those of the rats receiving thiouracil for two years.

Adenomas of the liver were seen in 4 rats receiving 0.0005 to 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide for one to two years and in 1 receiving 0.005 per cent thiouracil for two years. Fibroadenomas of the breast

¹³ Another apparently cancerous chromophobe adenoma was found in a rat not included in this study. The animal died after seven months of a diet containing 0.075 per cent of the selenium compound and 5 parts per million of sodium arsenite.

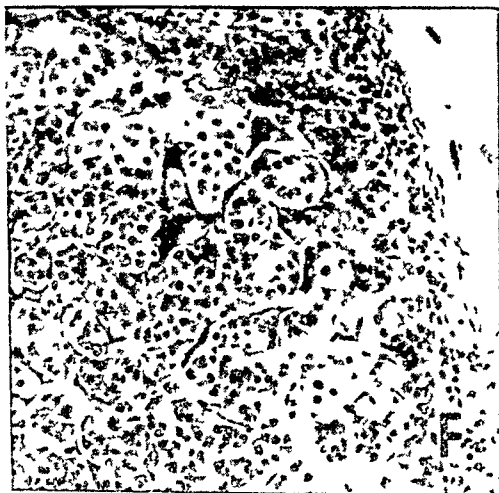
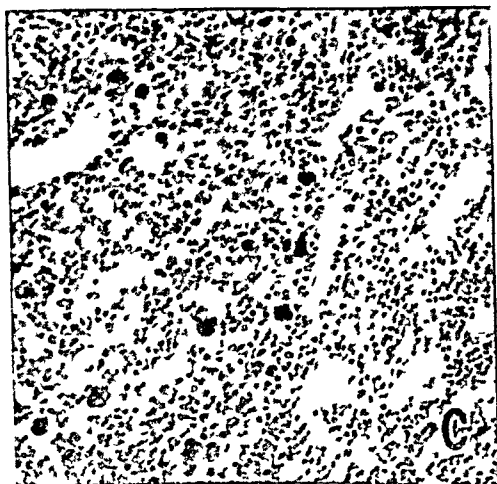
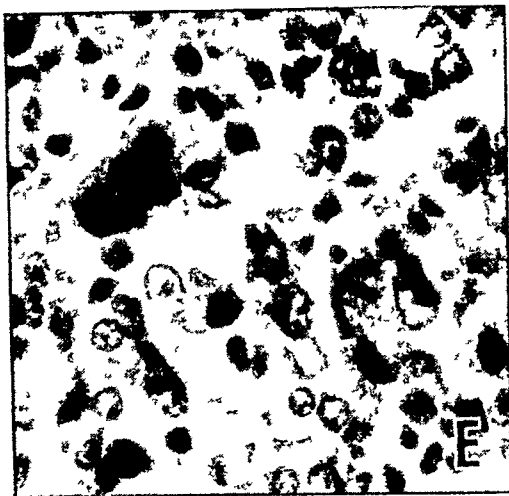
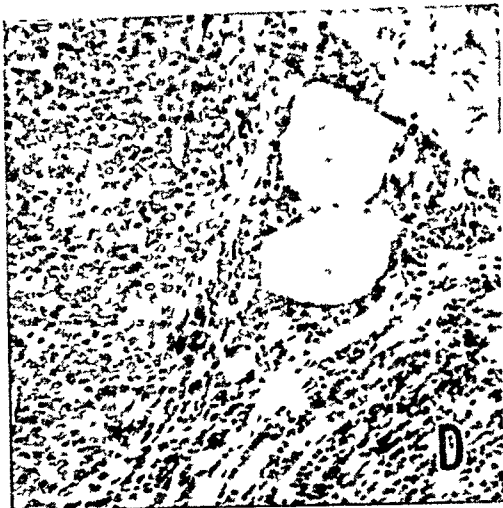
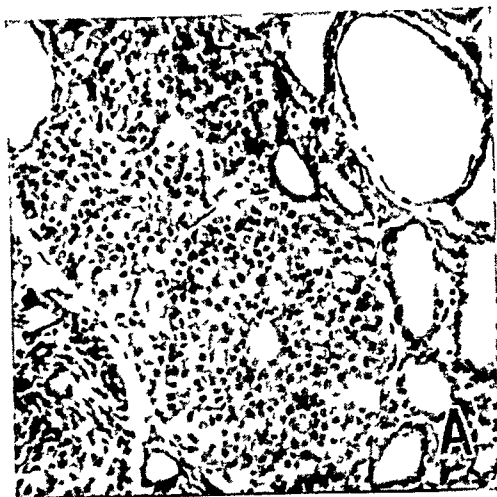


Fig 2—All sections were stained with hematoxylin and eosin. The magnification was $\times 200$ except that of *E*, which was $\times 500$. *A*, solid adenomas in thyroid gland of rat 18, fed selenium, 0.0005 per cent, for two years. *B*, simple adenoma in thyroid gland of rat 26, fed selenium, 0.075 per cent for seven months. *C*, benign chromophobe adenoma in anterior lobe of pituitary gland of rat 18, fed selenium, 0.0005 per cent, for two years. Note normal mother tissue at right. *D*, chromophobe carcinoma in anterior lobe of pituitary gland of rat 16, fed thiouracil, 0.005 per cent for two years. Note normal mother tissue at lower right. *E*, same as *D*, but magnified 500 instead of 200 times. Note the anaplasia of tumor cells. *F*, medullary adenoma infiltrating the cortex of an adrenal gland of rat 17, fed thiouracil, 0.01 per cent for two years.

were found in 1 rat fed 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide for two years. Widespread carcinomatosis was seen in a rat that died in the sixty-seventh week of 0.005 per cent bis-(4-acetaminophenyl) selenium dihydroxide treatment.

COMMENT

It is generally believed that the thyroid gland continues to enlarge macroscopically for periods as long as fifty days¹⁴. In rats fed a rape seed diet, however, its growth after the twentieth day merely paralleled the body growth¹⁵.

From our experiments it appears that the enlargement of the thyroid gland induced by antithyroid compounds is maintained indefinitely throughout the period of administration. The only exception occurred in a rat in which a chromophobe carcinoma of the anterior lobe of the pituitary gland developed. The rate of enlargement is greatest during the first few weeks of administration and gradually slows thereafter. Thiouracil when administered at a dietary level of 0.01 per cent for one to two years produced thyroid glands weighing three times as much as those of normal controls, when fed at this same level for ten days it produced glands only twice the normal size¹⁰. There appears to be no regression in the size of the thyroid gland after prolonged treatment.

It is also believed that the hyperplasia characteristic of the acute effects is maintained for as long as one hundred days¹⁴. In rats fed a rape seed diet, however, colloid reappeared in significant amounts in the follicles after the fiftieth day¹⁵. The fact that the "normal" thyroid tissue of a rat in which a carcinoma of the thyroid gland developed after twenty-two months of thiourea treatment was less responsive to thiourea than is usual was attributed to the cachexia and malnutrition from which the animal was suffering¹⁶.

In microscopic appearance the thyroid glands of the rats varied considerably. Some showed focal hyperplasia limited to the central portions, others revealed diffuse hyperplasia involving the whole gland. The minimal effective doses of the more potent antithyroid compounds and even maximal concentrations of the weakly acting para-aminobenzoic acid caused only focal hyperplasia, while larger doses of the more effective compounds caused diffuse goiters. It appears, therefore, that the intensity of antithyroid action determines whether the goiter will be diffuse or focal.

We¹⁰ have already reported that the hyperplasia seen in acute experiments was of the usual macrofollicular type. In the present study the hyperplasia seen after prolonged administration of antithyroid

14 Charipper, H. A., and Gordon, A. S. *Vitamins & Hormones* **5** 273, 1947.

15 Kennedy, T. H., and Purves, H. D. *Brit J Exper Path* **22** 241, 1941.

16 Purves, H. D., and Griesbach, W. E. *Brit J Exper Path* **27** 294, 1946.

compounds was of a microfollicular variety and resembled the microfollicular goiter seen in human patients by Smith and Gault¹¹

The transition to a microfollicular goiter was completed in most rats killed after thirty-five weeks of a diet containing thiouracil in concentrations of 0.005 per cent and 0.01 per cent. In 3 rats killed after one year, however, only the transitional picture from macrofollicular to microfollicular hyperplasia was seen. Similar changes were observed in rats receiving 0.1 per cent 2-aminothiazole.

The microfollicular change occurred also in glands showing focal hyperplasia. In a group of 6 rats receiving bis-(4-acetaminophenyl) selenium dihydroxide for one year, there were 4 whose thyroid glands showed transitional stages, the thyroid glands of the other 2, as well as that of a rat killed after two years of this treatment, had microfollicular goiters.

During the course of the microfollicular change the glands showing diffuse hyperplasia revealed the development of single or small groups of follicles considerably distended with thick colloid and lined with very low epithelium. These were seen as early as the fourteenth week of 0.005 per cent thiouracil but were developed best after one year of treatment, they were greatly reduced after two years of treatment. Such follicles were not observed in rats whose thyroid glands showed focal hyperplasia. Instead we found enlargement of the peripheral follicles which were not affected by the hyperplasia. These observations indicate that the distended colloid-containing follicles developing in the presence of diffuse hyperplasia are adaptation phenomena. The fact that they regress after development of the microfollicular stage suggests that the latter also is an adaptation phenomenon.

It is well established that benign and cancerous tumors of the thyroid gland develop in rats receiving antithyroid compounds for long periods.¹⁷

In the present study, adenomas were seen after seven months of a diet containing the selenium compound in a concentration of 0.075 per cent. One rat receiving 0.005 per cent of this compound for two years had a solid adenoma consisting of atypical thyroid epithelial cells whose benign or cancerous nature could not be established.

Studies by others have led to the conclusion that the changes in the pituitary gland coincided with those in the thyroid gland without the increase in weight. The basophilic cells rapidly increased in number and underwent hyalinization and vacuolation with the formation of "signet ring" cells, while the acidophilic cells decreased in number and underwent degranulation, the chromophobe cells were not changed.¹⁴

17 Dalton, A. J., Morris, H. P., and Dubnik, C. *Federation Proc.* **5**: 219, 1946. Seifter, J., Ehrlich, W. E., Hudyma, G., and Mueller, G. *Science* **103**: 762, 1946. Griesbach and others.¹ Purves and Griesbach.²

The development of "thyroidectomy cells" on a rape seed diet was maximal after fifty days and then declined so that in one hundred gland days the pituitary appeared essentially normal¹⁸ Rats receiving 0.1 per cent thiouracil for four months showed "fairly numerous thyroidectomy cells", those receiving 1 per cent thiourea had "enlarged chromophobic hypophyses without acidophilic cells"¹⁹

In contrast to this, the pituitary glands of our rats were in most instances considerably enlarged after antithyroid compounds had been fed for one year or longer Some of them showed no conspicuous microscopic changes, but most of the enlarged ones consisted chiefly of poorly granulated cells or chromophobes, thyroidectomy cells were no longer present After two years of antithyroid drug treatment 2 of 3 rats showed discrete chromophobe tumors, one a benign tumor and the other a lesion having the histologic characteristics of carcinoma The latter rat had received thiouracil at a dietary level of 0.005 per cent, and the former, bis-(4-acetaminophenyl) selenium dihydroxide at a level of 0.0005 per cent

Our finding that adrenal glands are smaller than normal in rats treated with effective doses of thiouracil and bis-(4-acetaminophenyl) selenium dihydroxide is in agreement with the findings of Leatham²⁰ for acute effects of thiourea and of Leblond and Hoff¹⁹ for chronic effects of it Kennedy and Purves,¹⁵ however, observed enlarged adrenal glands in rats on a rape seed diet

Since cortical adenomas of the adrenal glands are of frequent occurrence in old rats, we attach no great significance to finding them in our animals The presence of medullary adenomas infiltrating the cortices of a rat receiving for two years 0.01 per cent thiouracil is highly interesting, however, in view of the medullary hyperplasia observed by Bauman and Marine²¹ in animals treated with thiouracil

The failure to find changes in the gonads in the rats is in agreement with published observations,²² as is the finding of adenomas of the liver in selenium-treated and thiouracil-treated rats²³ The other tumors observed may have been of spontaneous rather than experimental occurrence, although a selenium effect may have been responsible, since bis-(4-acetaminophenyl) selenium dihydroxide contains 20 per cent selenium, which could possibly be released by breakdown of the compound

18 Griesbach, W. E. *Brit J Exper Path* **22** 245, 1941

19 Leblond, C. P., and Hoff, W. E. *Endocrinology* **35** 229, 1944

20 Leatham, J. H. *Endocrinology* **36** 98, 1945

21 Bauman, E. J., and Marine, D. *Endocrinology* **36** 400, 1945

22 Seegar Jones, G. E., Delfs, E., and Foote, E. E. *Endocrinology* **38** 337, 1946

23 Fitzhugh, O. G., Nelson, A. A., and Bliss, C. I. *J Pharmacol & Exper Therap* **80** 289, 1944; Fitzhugh, O. G. *Science* **108** 676, 1948

SUMMARY

Rats receiving antithyroid compounds for varying periods up to two years had significant changes in the thyroid gland and, in some instances, in other endocrine glands

The weight of the thyroid gland did not regress. Low doses of the compounds caused hyperplasia only of the central portions of the gland, while a larger intake caused diffuse hyperplasia. The macrofollicular hyperplasia characteristic of acute effects changed to microfollicular. The change was completed in some experiments as soon as the thirty-fifth week of intake of antithyroid drug. In diffuse goiters it was preceded by focal restoration of colloid. The microfollicular change and the focal restoration of colloid are interpreted as adaptation phenomena. Adenomas of the thyroid gland were also observed. They occurred earliest with administration of bis-(4-acetaminophenyl) selenium dihydroxide.

The pituitary glands of many of our rats showed hyperplasia. Thyroidectomy cells were absent after one year or later. The enlargement was due chiefly to multiplication of poorly granulated cells or chromophobes. Two rats showed chromophobe adenomas, one of which was cancerous.

The adrenal glands were atrophic in most instances. In 1 rat medullary adenomas developed in the cortices of both glands.

BILATERAL EXTENSIVE FOCAL ISCHEMIC ATROPHY OF THE KIDNEYS

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AND

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LESIONS resulting from occlusion of the branches of both renal arteries have been described in bilateral cortical necrosis of the kidneys¹ and as part of the systemic manifestations of lupus erythematosus disseminatus,² scleroderma,³ rheumatic fever,⁴ periarteritis nodosa⁵ and other diseases⁶. The basic causal factor in all is related in some way to injury of the arteries. The effects on the renal tissues depend on the extent of distribution and the completeness of the occlusions. Accordingly, large or small portions of the kidneys are involved, the regions affected are single and large or multiple and small, and when

From the Henry Baird Favill Laboratory, St Luke's Hospital

1 Duff, G L, and Murray, E G D *Am J M Sc* **201** 428, 1941 Weaver, R G, and von Haam, E M M *Arch Int Med* **63** 1084, 1939 Davidson, J Tr *Edinburgh Obst Soc* **89** 101, 1929-1930

2 (a) Klemperer, P, Pollack, A D, and Baehr, G *Arch Path* **32** 569, 1941 (b) Bunim, J J *Ann Int Med* **13** 1399, 1940 (c) Mook, W H, Weiss, R S, and Bromberg, L K *Arch Dermat & Syph* **24** 786, 1931 (d) Libman, E, and Sacks, B *Arch Int Med* **33** 701, 1924 (e) Contratto, A W, and Levine, S A *New England J Med* **221** 602, 1939 (f) Jarko, S *Bull Johns Hopkins Hosp* **59** 262, 1936 (g) Jager, B V *Arch Dermat & Syph* **46** 362, 1942 (h) Dine, C H, and Wilson, C S *Am J Med* **1** 568, 1946

3 (a) Talbott, J H, Gall, E A, Consolazio, W V, and Coombs, F S *Arch Int Med* **63** 476, 1939 (b) Weiss, S, Stead, E A, Jr, Warren, J V, and Orville, T *ibid* **71** 749, 1943 (c) Pollack, A D *Arch Path* **29** 859, 1940

4 (a) Hutton, R L, and Brown, C R *Ann Int M* **20** 85, 1947 (b) Bruetsch, W L *J A M A* **134** 450, 1947 (c) van der Horst, L *Digest Neurol & Psychiat, Inst of Living* **15** 399, 1947

5 (a) Singer, H A *Arch Int Med* **34** 865, 1927 (b) Manges, M, and Baehr, G *Am J M Sc* **162** 162, 1921 (c) Lamb, A R *Arch Int Med* **14** 481, 1914 (d) Keegan, J J *ibid* **36** 189, 1925 (e) Spiegel, R *ibid* **58** 993, 1936 (f) Higgins, W H *South M J* **39** 453, 1946 (g) Pettit, H *Am Pract* **1** 333, 1947 (h) Massachusetts General Hospital Case, no 32381, *New England J M* **235** 441, 1946 (i) Massachusetts General Hospital Case, no 33181, *ibid* **236** 670, 1947

6 (a) Leiter, L *J A M A* **111** 570, 1938 (b) Fishberg, A M *Arch Int Med* **40** 80, 1927 (c) Derick, C L, and Hass, G M *Am J Path* **11** 291, 1935 (d) Massachusetts General Hospital Case, no 23041, *New England J Med* **216** 170, 1937

the occlusions initially are complete or extensive, renal function is reduced rapidly, death occurs, and the renal changes observed are those of acute necrosis. When the initial phase of the process of occlusion does not cause a sudden loss of functional renal tissues incompatible with living, other retrogressive changes occur in the involved tissues, such as atrophy, and in the portions with adequate blood supply, parenchymatous changes in the order of nephrosis. Kidneys with changes of this kind are herein described.

REPORT OF A CASE

A white woman aged 35 years had a migratory polyarthritis in the fifth month of her second pregnancy. The urine then contained only a trace of albumin, leukocytes and occasional erythrocytes. Shortly before she was delivered, her blood pressure was 162 systolic and 110 diastolic, and she had slight albuminuria. A full term live baby was born spontaneously. On the third day post partum the urine had a specific gravity of 1.016, a trace of albumin, no sugar, a few leukocytes and erythrocytes and many granular casts. Her blood pressure rose to a maximum of 184 systolic and 120 diastolic. Thirty-three days post partum she returned to the hospital because of cough, pain in the chest, dyspnea, hypertension, headaches and signs of cardiac decompensation. Two days later the sputum was stained with blood, and the right leg gave evidence of occlusion of the femoral artery. The urine now contained much albumin, many leukocytes and erythrocytes, and granular, waxy and cellular casts. The blood had 3,700,000 erythrocytes and 14,500 leukocytes per cubic millimeter. The following day she was transferred to the service of Dr. John T. Reynolds at St. Luke's Hospital. Circulation to the left leg was now also impaired. Decompensation of the heart was marked, and bilateral hydrothorax was present. The leukocytes were 26,150 per cubic millimeter, the clotting activity of the blood was 100 per cent, and the nonprotein nitrogen of the blood was 91 mg per hundred cubic centimeters. On the second day, embolectomy of the right femoral artery was done, and spinal anesthesia was continued after the operation in the hope of improving circulation in the lower extremities. The patient continued in progressive cardiac and renal failure with decreasing urinary output and increasing evidence of uremia. She died in a convulsion on the third postoperative day and forty days post partum.

The essentials of the anatomic diagnosis of the postmortem examination (head, neck and trunk) were: subacute purpurperal endometritis of the uterus, thrombosis of the veins of the broad ligament, mural thrombi of the left and right ventricles and auricles of the heart, bilateral extensive focal atrophy of the cortex of the kidneys, obturator thrombi of the left renal vein and of one of the two right renal veins and of the left and right internal iliac, left femoral and right profunda femoris arteries, recent femoral embolectomy wound of the right thigh, bilateral hydrothorax.

The uterus was slightly enlarged. Large portions of the endometrium of the upper part of the body were absent, and here the lining surface was granular. The endometrium remaining was gray and yellow, frayed, and 4 mm thick. Veins of both broad ligaments were thrombosed. The heart weighed 380 Gm. In the right auricular appendage and in a pouch on the left side of the interatrial septum were small gray-red thrombi. Behind the posterior leaflet of the tricuspid valve were two gray mural thrombi 1 cm in diameter and elevated 3 mm. Between the columnae carnae of the right ventricle were others. The left ventricle was dilated, and along the septum in front was a partially liquefied red-brown mural thrombus.

3 cm in diameter and 8 mm thick. The leaflets of the heart valves had no changes. Cultures of the heart blood, the spinal fluid and the pericardial fluid were sterile.

The kidneys weighed, respectively, 155 and 165 Gm. The capsule of each stripped easily from a finely granular red-brown surface with scattered plateaus of a gray-yellow tissue, elevated 2 to 3 mm above the red-brown portions (fig 1). These elevated regions varied from 1 mm to 2 cm in maximum diameter and comprised about 45 per cent of the external surface. The gray-yellow tissues reached wedge-like through the cortex into the columns of Bertini, but not into the medulla, and

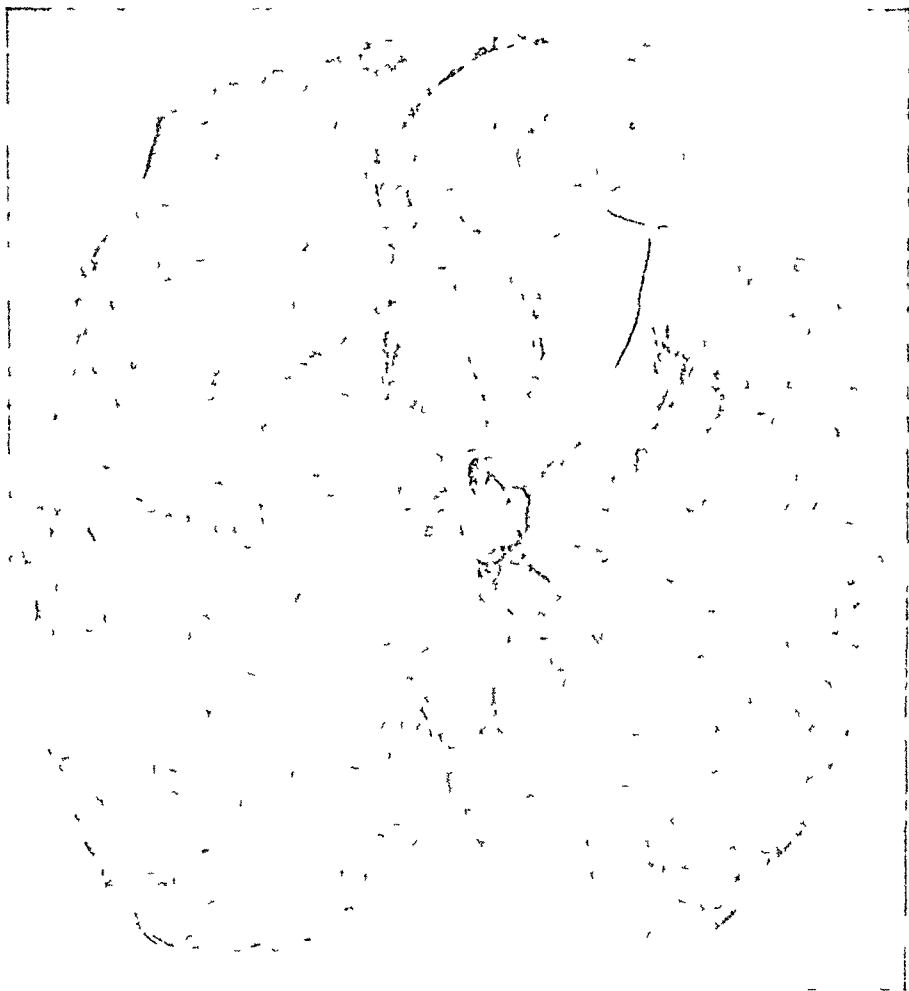


Fig 1—Subcapsular surface of the right kidney showing the depressed red-brown regions of cortical atrophy and the distribution of the plateau-like portions of gray nephrotic tissue.

had indistinct markings (fig 2). The red-brown depressed tissues of the cortex were 5 mm thick at the base of a pyramid. The left renal vein had an obturator thrombus 2 cm long, the right kidney had two veins and the one to the upper pole contained an obturator thrombus which extended into the smaller branches. The renal pelves were not unusual.

Each pleural space contained about 800 cc of a clear yellow fluid. The lungs were moderately hyperemic and edematous. Lymph nodes in the hilus of the left

lung had small encapsulated foci of caseous tissue. The liver weighed 2,250 Gm. Under the capsule were about a dozen gray tubercles, all less than 2 mm in diameter. Surfaces made by cutting the liver were pale, red-brown, with distinct lobular markings and moderate fatty changes. The spleen weighed 140 Gm and had about six small gray tubercles 2 mm in diameter beneath the capsule and in the parenchyma. The lining of the stomach had a few petechial hemorrhages.

Histologic preparations of the endometrium of the uterus showed definite necrosis, which extended slightly into the myometrium. In many large arteries



Fig 2—Surfaces made by hemisectioning the left kidney, showing the wedge-shaped regions of gray nephrotic tissues extending through the cortex

of the myometrium the intima was so thickened by fibrous tissues that the lumen was almost occluded. Others contained partially organized thrombi, and about them were exudates of lymphocytes and a few polymorphonuclear leukocytes. Many veins and some small arteries of the broad ligament also had thrombi in various stages of organization.

The mural thrombi of the left ventricle of the heart were partially organized. No masses of bacteria were found. In the underlying myocardium were a few lymphocytes and occasional polymorphonuclear leukocytes. Deeper in the myocardium were a few clusters of mononuclear cells about the blood vessels.

The renal tissues had two distinct patterns of structure. In the elevated portions, the glomeruli were large, and none was hyalinized (fig 3 *A*). The afferent arterioles were not thickened, and the lumens of the vessels were patent. The

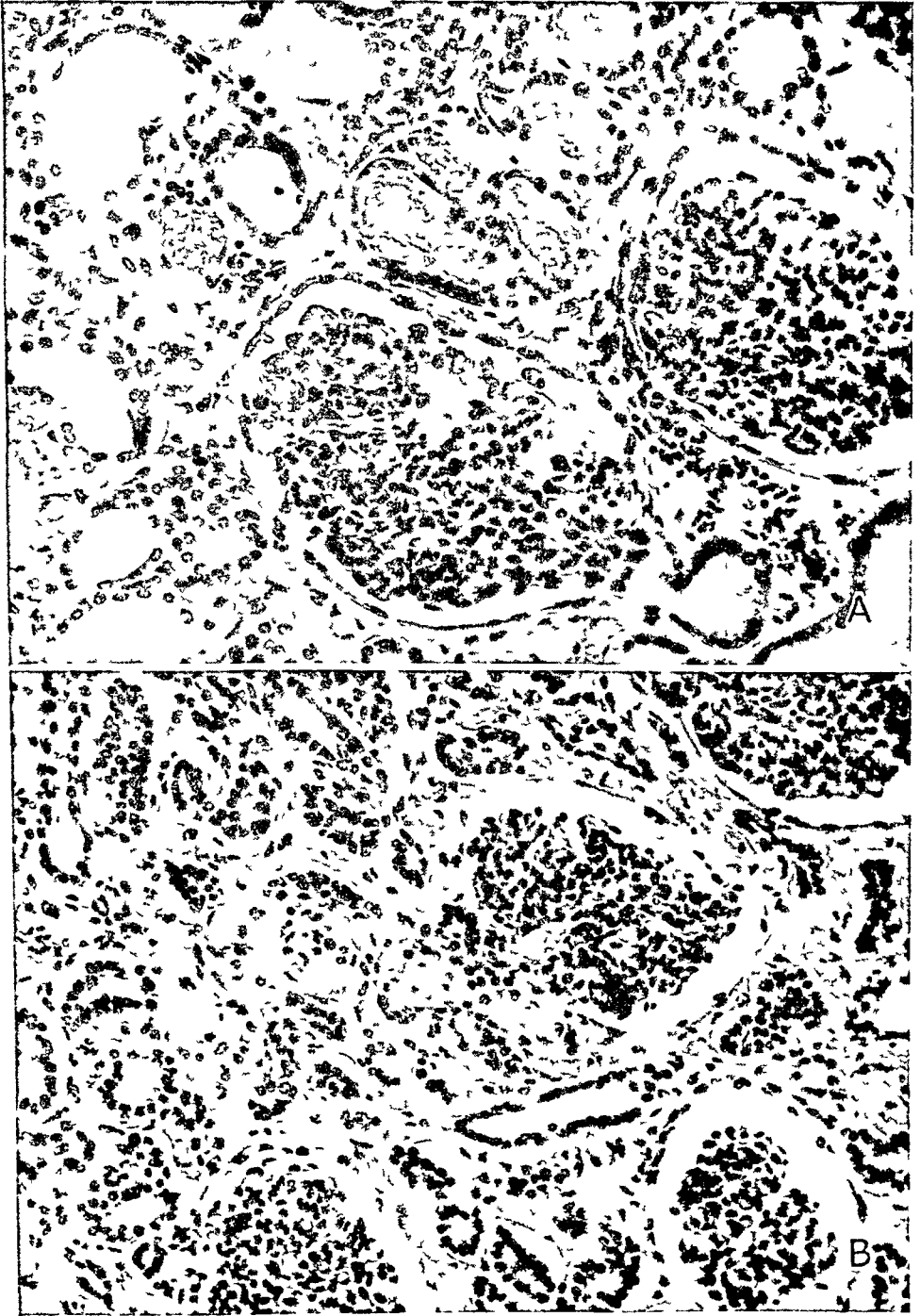


Fig 3—Photomicrograph illustrating the structure of (*A*) the elevated gray tissues of the kidney and (*B*) the depressed red-brown portions, $\times 198$

efferent arterioles were dilated with blood cells. Occasional intralobular arteries had a slight perivascular accumulation of lymphocytes, plasma cells and a few polymorphonuclear leukocytes. In sections cut serially some of the interlobular

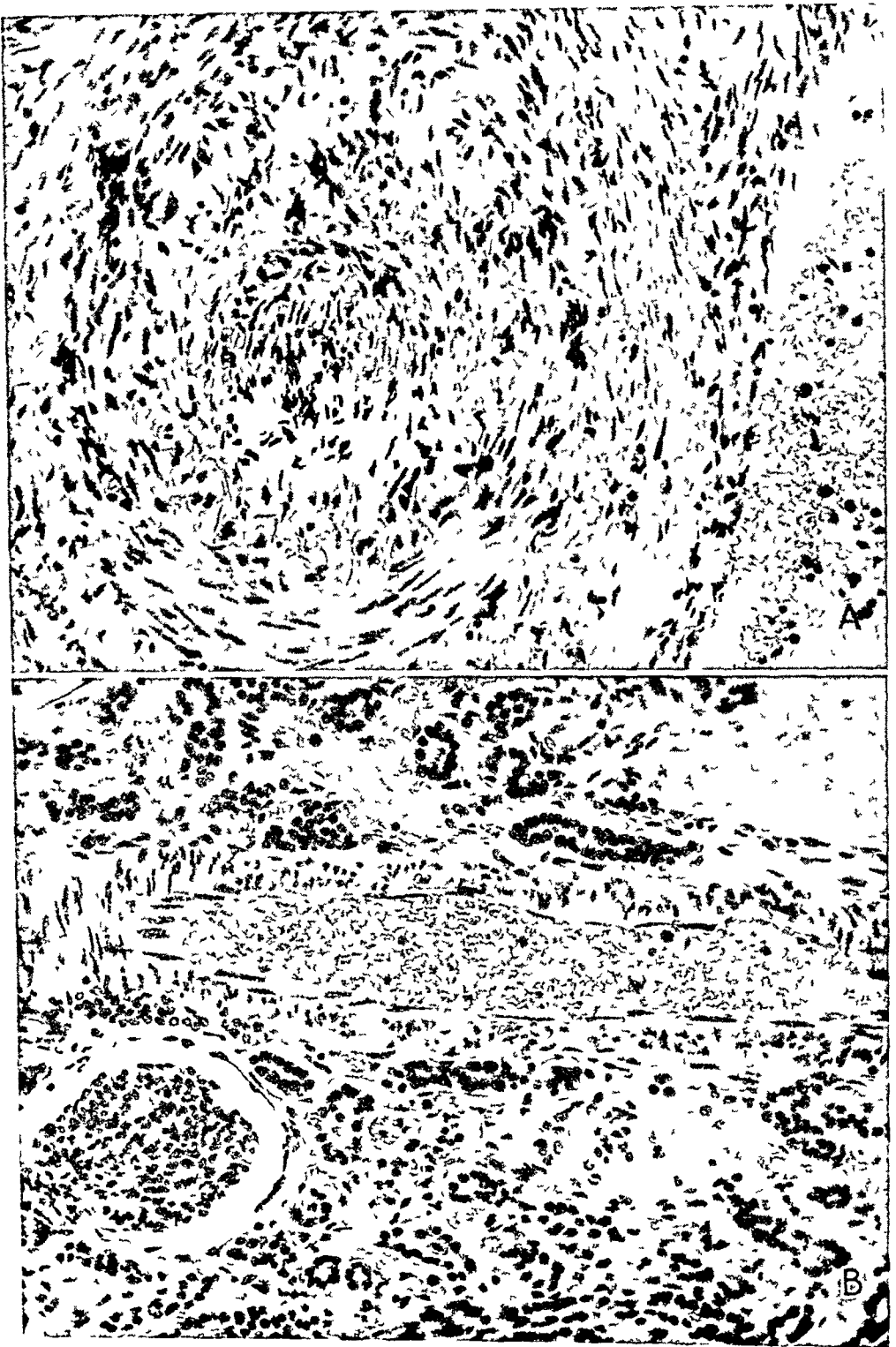


Fig 4—*A*, photomicrograph of an interlobular artery illustrating the extensive fibrous tissue thickening of the intima and the greatly reduced lumen
B, photomicrograph of an intralobular artery in an atrophic portion of the kidney, illustrating the muscular wall and the widely patent lumen

arteries had lumens partially occluded by fibrous tissue thickenings of the intima but had no perivascular inflammatory exudates and the internal elastic membrane was intact. A few of the convoluted tubules had flattened lining cells, but the others had columnar cells whose cytoplasm contained fine lipid deposits and distinct nuclei. Within the lumens of these tubules were granular precipitates. The collecting tubules had the usual low columnar lining cells, and their lumens contained amorphous precipitates. The depressed portions of the cortex contrasted sharply (fig 3 B). The glomeruli here were shrunken, the tufts were contracted and more cellular, and their capillaries were small. A few glomeruli were hyalinized. Bowman's capsule was unchanged. At all levels of the nephron the tubules were atrophic and in appearance resembled those of a fetal kidney. Accordingly, the number of atrophic glomeruli and tubules per unit area was greatly increased. The interstitial tissues were not appreciably increased. The afferent arterioles also had thin walls, and some of the intralobular arteries had perivascular collections of inflammatory cells but patent lumens. Many of the arcuate and interlobular arteries had fibrous thickenings of the intima ranging to complete occlusion (fig 4 A). Some were recanalized, and most of them had only a few mononuclear leukocytes in the wall. The intralobular branches had muscular walls but no occlusion (fig 4 B). Larger branches of the renal vein also had lumens occluded by organized thrombi.

There were a few small hemorrhagic infarcts in the lungs. The aorta showed slight fatty changes. A few of the vessels in the pancreas had small perivascular collections of lymphocytes. The blood vessels of other viscera were unchanged.

The changes of the kidneys were considered to be the result of disseminated fibrous tissue occlusions of the branches of the renal arteries which supplied the depressed red-brown regions of tissue and caused anemic atrophy of the parenchyma. The elevated gray-yellow tissues were regions spared or at least had a blood supply adequate to maintain structure.

Eight reports of similar focal atrophy of the kidney have been found in a review of the English literature. Mallory^{6a} observed it in the kidneys of a man aged 48 years who died of coronary thrombosis. The medium-sized arteries of the kidneys had prominent hyaline fibrous tissue thickenings which greatly decreased the lumen. Mallory could not determine whether the changes were due to sclerosis or to endarteritis. This man had been treated with arsenical preparations for syphilis. Leiter^{6a} described similar changes in the kidneys of a man aged 40 years in whom, after arsenical treatment for syphilis, hypertension rapidly developed. Although typical syphilitic cerebral endarteritis was present and spirochetes were demonstrated in necrotic duodenal tissues, Leiter was reluctant to consider the renal lesions as syphilitic, because they differed from the syphilitic nephritis reported by Volhard,⁷ Wohlwill⁸ and Rich.⁹ He considered the possibility that the changes were an end result of a thrombotic process though not of visceral thromboangitis obliterans. The renal lesions described by Talbott and associates, in a patient with

7 Volhard, F. *Handbuch der inneren Medizin*, Berlin, Julius Springer, 1931, vol 2, pt 2, p 1541.

8 Wohlwill, F. *Zentralbl f inn Med* 47 1066, 1926.

9 Rich, A. R. *Bull Johns Hopkins Hosp* 50 357, 1932.

dermatomyositis accompanied by scleroderma and calcinosis, were multiple regions of atrophy of the cortex. The intralobular arteries supplying these portions had great narrowing of the lumens caused by fibrous tissue thickenings of the intima. Some vessels had necrosis of the intima and inner portion of the media. Klemperer, Pollack and Baehr^{2a} observed similar renal lesions in 3 of 35 cases of lupus erythematosus, but in appearance the lesions of the kidneys, they stated, resembled the focal scars of malignant nephrosclerosis. It is not clear from their description whether the changes in the kidneys were as extensive as those in the kidneys described in our report. However, they regarded them as due to vascular occlusions. Bunim^{2b} also observed focal renal atrophy in lupus erythematosus, but in the patient, a woman aged 20 years, the advanced parenchymal atrophy was present only in the right kidney. Here large numbers of the interlobar and arcuate arteries had lumens partially or completely obliterated by dense connective tissue thickening of the intima and the elastic membrane and media were unaltered. No vascular lesions were seen in the left kidney. Hutton and Brown^{4a} described rheumatic endarteritis of the renal vessels of a woman aged 31 years. The kidneys had not only regions of atrophy but also hyaline infarcts. The dense proliferations of collagenous fibrous tissue of the intima occurred not only in portions of the arcuate and larger arteries but also in the interlobular arteries. The myocardium contained Aschoff bodies and revealed endarteritis.

Other reports of similar lesions of the kidneys may have been published in foreign literature or in articles whose titles indicate neither this pathologic disorder nor the syndromes with which it has been found. A similar atrophy from intimal proliferation but usually associated with medial necrosis, aneurysmal dilatations and rupture of arteries producing also recent infarcts and hemorrhages has been described in periarteritis nodosa. Singer^{5a} described the lesions in the kidneys of a man aged 57 years who died of chronic nephritis and hypertension. The capsular surface of the right kidney had gray elevations separated by gray-red depressed tissues. The blood vessels had thickened walls. Regions of atrophy of the cortical tissues alternated with regions in which the parenchyma appeared uninvolved. Although small aneurysms with medial necrosis were seen elsewhere, the arteries of the kidneys were not dilated. Other borderline examples have been described, and those in which an endarteritis produced hyaline scars. The latter occurred in a patient with lupus erythematosus observed by Mook and associates.^{2c}

Atherosclerotic stenosis of a renal artery which greatly reduces the volume of blood flow causes atrophy of an entire kidney. This was observed in the body of a woman aged 62 years whose right renal artery had an ostium so narrowed by atherosclerosis that a 2 mm probe was passed with difficulty. The shrunken right kidney weighed only 55 Gm ,

the left, with an unimpeded blood supply, weighed 145 Gm. The tissues of the right kidney were atrophic and like those in the red-brown depressed regions of the kidney described.

Probably the small number of published reports of these changes occurring in both kidneys indicates that they occur rarely. Those recorded have been associated with various diseases. In our case the lesions of both kidneys seem to be part of a complication of pregnancy in which puerperal endometritis, systemic venous and arterial thrombosis and thrombosis of the endocardium occurred. Cultural studies of the body fluids at the time of the necropsy, as well as a search of sections of the endocardial thrombi, failed to disclose bacteria. These negative results, however, do not exclude the presence of an infectious agent.

SUMMARY

Bilateral focal ischemic atrophy of the cortex of the kidneys caused by fibrous tissue or thrombotic occlusions of the intrinsic branches of the renal arteries has been reported in patients with lupus erythematosus disseminatus, scleroderma, rheumatic fever, periarteritis nodosa and possibly syphilitic endarteritis of the kidney. The disorder, according to our study, may occur as a complication of pregnancy.

Grossly, the atrophic portions are depressed red-brown tissues of the cortex, which contrast sharply with plateau-like regions of gray nephrotic renal tissues. The ischemic atrophy of the cortical tissues of the kidney results from a diminished flow of blood in the affected portions. The parenchymal changes in the atrophic portions are chiefly a decrease in the size of the various segments of the nephrons, which revert finally to structures resembling those in a fetal kidney.

The thrombotic and fibrous tissue occlusions of the intrinsic branches of the renal arteries are due to injury of these vessels. The nature of the noxious agent is not known, and may not be the same in the various diseases in which these lesions of the kidneys have been observed.

Renal functions, reduced progressively by the vascular lesions, become inadequate, symptoms of renal failure appear and death in uremia follows.

INFLUENCE OF AGE ON MAMMARY GROWTH AND INVOLUTION IN MALE MICE TREATED WITH ESTROGEN

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THE SUSCEPTIBILITY of male mice to estrogen-induced mammary cancer is partly determined by the age of the animal at the beginning of the treatment¹. The lower incidence of such cancer in older animals has been attributed to a decreased responsiveness of the breast tissue and to an inhibiting effect exerted on mammary growth by the male sex hormone (androgen)¹. The present report deals with the microscopic changes observed in the noncancerous mammary glands of castrate and noncastrate mice receiving an estrogen at various ages. It was thought that such an investigation might throw some additional light on the role played by the age factor in estrogen-induced mammary growth.

MATERIAL AND METHODS

Sixty-five male mice of the closely inbred strain C3H, raised in our laboratory, were castrated at the age of 3 to 4 weeks. Twenty-three of these animals were given an injection of 0.03 mg of alpha estradiol benzoate,² dissolved in sesame oil, once a week for five months from the age of 1 month on (younger age group), the remaining 45 animals received the same treatment from the age of 4 months on (older age group). Fifty-eight mice with intact testicles were given a similar course of injections, 26 animals from the age of 1 month on (younger age group) and 32 from the age of 4 months on (older age group). Details concerning the arrangement of the experiments, the technic employed, and the incidence of mammary cancers and lymphoid tumors in these mice have been published elsewhere^{1b,c}. The present microscopic examination revealed two additional small carcinomas, one in a castrate and one in a noncastrate of the younger age group.

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¹ Loeb, L. (a) *Biol Sympos* **11** 197, 1945. (b) Silberberg, M., and Silberberg, R. *Proc Soc Exper Biol & Med* **69** 438, 1948, (c) *Arch Path* **47** 340, 1949.

² The Schering Corporation, Bloomfield, N. J., supplied the estrogen used, progynon-B®.

The resulting change in the tumor incidence, however, does not warrant a modification of the conclusions arrived at previously on the basis of the gross observations^{1b}

All animals except those in which palpable cancers or lymphoid tumors developed had been allowed to live to the end of their natural life. Not all of the available material could, however, be utilized. Some animals found dead had to be excluded from the microscopic study because of autolytic changes. Moreover, in order to satisfy the purpose of the present investigation, we could compare the breast tissues only of those animals that had lived for similar periods of time after discontinuation of the treatment.

Altogether, in the younger age group 26 mice (11 with intact testicles and 15 castrates) were used, in 4 of the former and 7 of the latter mammary cancer had developed. In the older age group 27 mice (9 with intact testicles and 18 castrates) were examined, mammary cancer had developed in 1 of the former and 7 of the latter (see tables 1 and 2). Usually 4 mammary glands were removed at necropsy, fixed in 4 per cent solution of formaldehyde on blotting paper to prevent curling, sectioned at several levels at 5 microns and the sections stained with hematoxylin and eosin.

MICROSCOPIC OBSERVATIONS

NONCASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF ONE MONTH ON

Animals in Which No Breast Cancer Had Developed—At the age of 8 to 11 months, that is, two to five months after discontinuation of the estrogenic treatment (fig 1A), ductal proliferation and secretion were accentuated, and in the acini, hyperplasia and hypertrophy, as well as secretion, were marked. Varying amounts of connective tissue were seen between the acini. At the age of 12 to 14 months, that is, six to eight months after the last injection (fig 1B), growth processes and secretion were definitely decreased as compared with the earlier stages in both ducts and acini, many acini were broken up, and there was abundant connective tissue surrounding the remaining epithelial elements. At the age of 15 months or over, that is, nine or more months after cessation of the treatment, the ducts and acini showed no hyperplastic or hypertrophic changes, and the resting state had recurred in 2 of 3 mice. Only an occasional globule of secreted material in a collapsed duct or in an acinus and a marked increase of fibrous tissue indicated that a previous stimulation of growth had taken place. In 1 animal, 17 months of age, active stimulation of the acinous growth was found, with the development of only small amounts of connective tissue.

Animals in Which Breast Cancer Had Developed—The grossly noncancerous mammary glands showed advance stimulation of growth and pronounced secretion in the proliferating and dilated ducts and acini. There was neither involution nor decrease in the process of growth with an increasing interval after the last injection. A 14 month old mouse showed a small papillary cystadenoma in one mammary gland and an intraductal papilloma in another gland.

NONCASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF FOUR MONTHS ON

Animals in Which No Breast Cancer Had Developed—In ducts and acini of 8 to 11 month old mice there was but slight evidence of hyperplasia and hypertrophy of the epithelium. Some secretory globules were present, and the periacinous

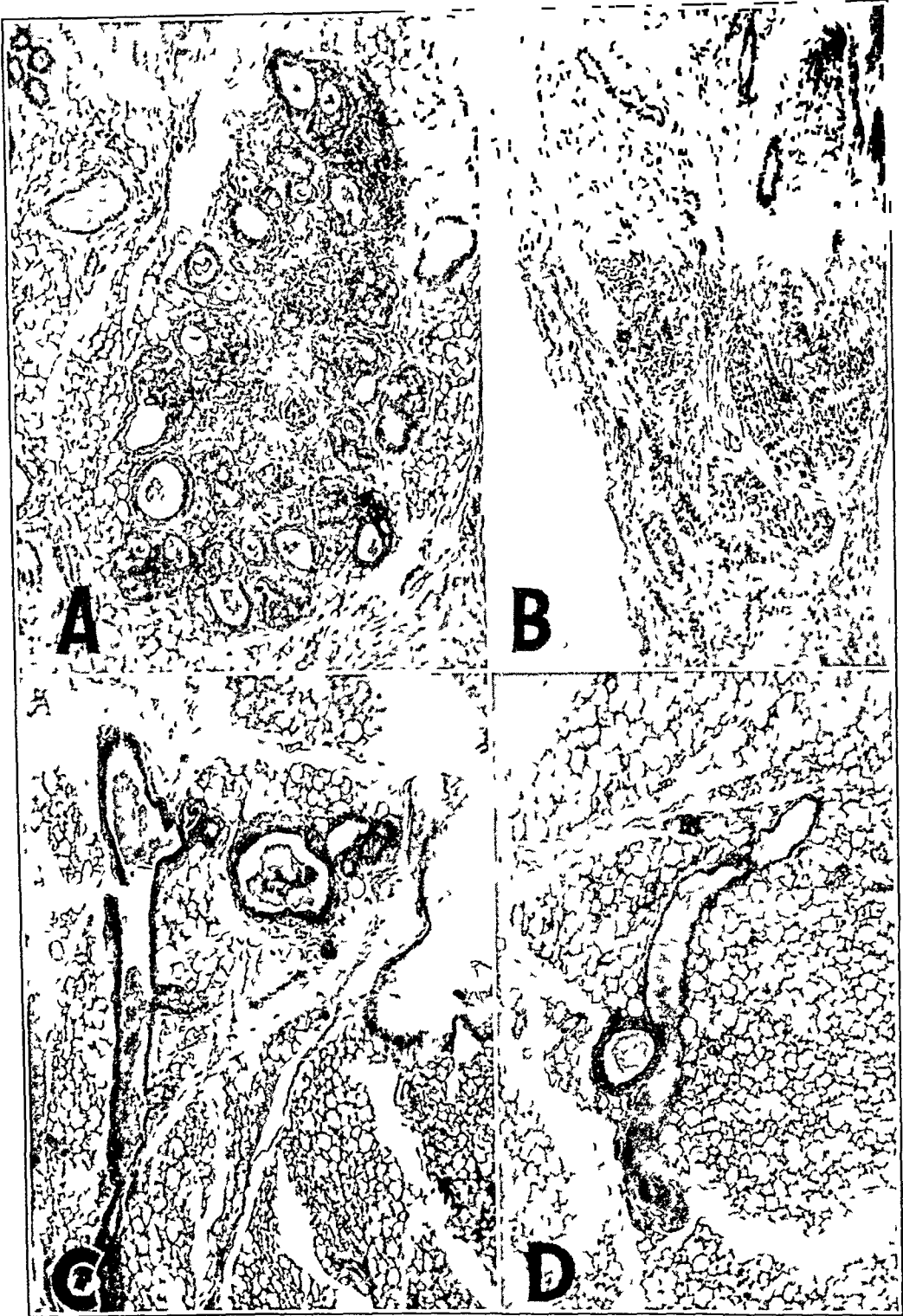


Fig 1—Sections through mammary glands of male mice of strain C3H *A*, noncastrate male, 9 months old, treated with estrogen from the age of 1 month, tissue taken three months after the last injection, $\times 70$ Ductal and acinous hyperplasia and secretion are seen There is a moderate amount of connective tissue inside the lobule

B, noncastrate male, 14 months old, treated with estrogen from the age of 4 months, tissue taken eight months after the last injection, $\times 70$ A few ducts and isolated remnants of acini are seen The lobular arrangement of the collapsed connective tissue can still be recognized, besides interlobular bands of dense connective tissue

C, noncastrate male, 12 months old, treated with estrogen from the age of 4 months, tissue taken three months after the last injection, $\times 70$ A few dilated ducts show some budding and contain secretion

D, noncastrate male, 17 months old, treated from the age of 4 months, tissue taken eight months after the last injection, $\times 70$ Note resting duct containing some secretion

connective tissue was increased, particularly in 1 of the 3 animals. In mice 12 to 14 months old, that is, three to five months after the last injection, the few acini present were in a resting state. The dilated ducts were lined by somewhat hypertrophic epithelium and contained some secretions (fig 1 C). There was no increase of connective tissue. In the animals 15 months of age or older, in which the injections had been discontinued for six or more months before death, there was but slight dilatation of an occasional duct (fig 1 D), which was surrounded by abundant adipose tissue. Acini were not found except in one mammary gland of 1 animal 20 months of age. In the latter a small adenomatous nodule was noted. In another mammary gland of the same mouse an intraductal papilloma was observed.

Animals in Which Breast Cancer Had Developed—In the single animal in which a mammary cancer had developed at the age of 17 months, the ducts and acini of the grossly noncancerous mammary glands were distinctly hyperplastic and hypertrophic. One breast contained a cystadenoma of microscopic size. There was no evidence of involutionary changes.

CASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF ONE MONTH ON

Animals in Which No Breast Cancer Had Developed—In 8 to 11 month old mice, in which the estrogenic treatment had been discontinued since the age of 2 to 5 months (fig 2 A), proliferation of both ducts and acini and secretion were advanced. In 1 animal 11 months of age there was slight fibrosis around the acini. At the age of 12 to 14 months (fig 2 B), that is, six to eight months after cessation of the injections, ductal and acinous proliferation were of about the same order as at the earlier ages. The acini were, however, more dilated, and the epithelium was slightly lower than before. In mice 15 months of age or older the growth of the acinous epithelium was decreased, and the ducts had begun to collapse. The amount of secretion was likewise diminished, whereas the periacinous connective tissue was slightly increased.

Animals in Which Breast Cancer Had Developed—The grossly noncancerous mammary glands showed at all ages active secretion and advanced stimulation of growth of both ducts and acini. In 3 animals, 9, 14 and 16 months old, microscopic benign intraductal papillary tumors were observed. An additional animal, 17 months old, had a carcinoma of microscopic size in one mammary gland. Involutionary changes were missing or slight at best.

CASTRATE MICE TREATED WITH ESTROGEN FROM THE AGE OF FOUR MONTHS ON

Animals in Which No Breast Cancer Had Developed—In the single mouse examined at 10 months of age, that is, one month after the last injection, epithelial proliferation was quite marked in ducts and acini, and there was abundant secretion. No involutionary changes were noted. In animals 12 to 14 months of age, in which the injections had been discontinued for three to five months, respectively (fig 2 C), there were clusters of small acini scattered in the adipose tissue. The lining epithelium was low cuboidal, and secretion was present. The ducts showed some stimulation of growth. In most of the animals 15 months of age or older, in which injections had been discontinued for six or more months

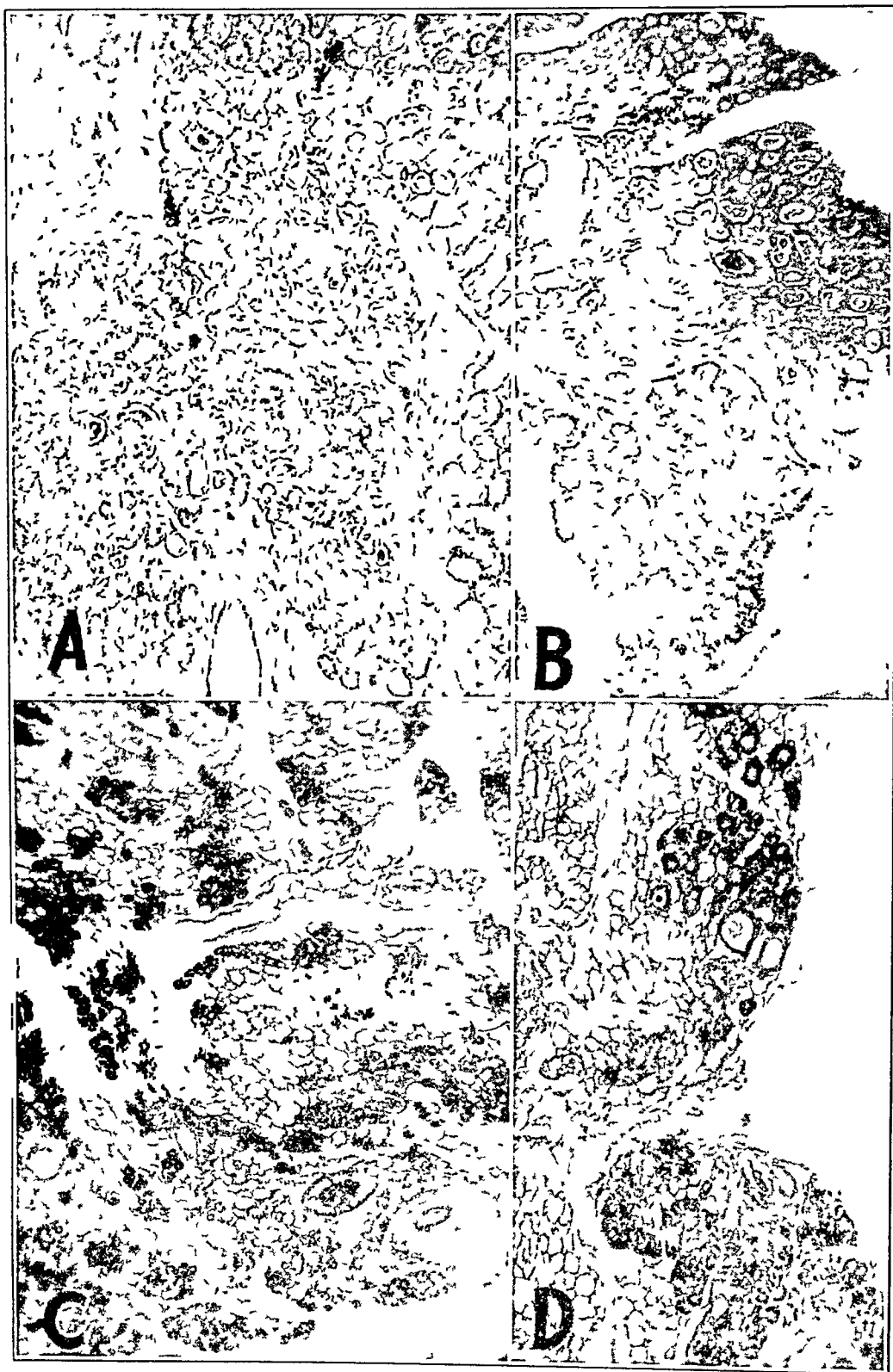


Fig 2—Sections through mammary glands of male mice of strain C3H *A*, castrate male, 9 months old, treated with estrogen from the age of 1 month, tissue taken three months after the last injection, $\times 70$ This is a large mammary nodule composed of densely packed acini The lumens of the acini are lined by hyperplastic epithelium and contain varying amounts of secretions Little connective tissue is present between the acini

B, castrate male, 14 months old, treated with estrogen from the age of 1 month, tissue taken eight months after the last injection, $\times 70$ Mammary nodules are seen, composed of densely packed acini containing secretions and lined by hyperplastic epithelium There is no evidence of involutionary changes

C, castrate male, 12 months old, treated with estrogen from the age of 4 months, tissue taken three months after the last injection, $\times 70$ Scattered groups of small acini are seen in the fat tissue

D, castrate male, 17 months old, treated with estrogen from the age of 4 months, tissue taken eight months after the last injection, $\times 70$ Few clusters of small acini and some ducts are seen There is no evidence of involutionary changes

before death (fig 2D), ductal and acinous growth was accentuated. A few dilated acini contained secreted material. The epithelial lining of ducts and acini was cuboidal and inactive. In 2 of the 8 animals the acinous proliferation was more diffuse and more active than in the rest of the mice. In 1 of these 2 animals

TABLE 1—*The Degrees of Proliferation, Secretion and Involutionary Changes Observed in the Mammary Glands of Noncastrate Male Mice*

Age at death, mo Interval since last injection, mo	Younger Age Group (Treated with Estrogen from the Age of 1 Month on)						Older Age Group (Treated with Estrogen from the Age of 4 Months on)					
	8 11		12 14		15+		8 11		12 14		15+	
	2 5		6 8		9+		0 2		3 5		6+	
	No Cancer	Can cer	No Cancer	Can- cer	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer
Cases	2	2	2	1†	3	1	3	0	2	0	3†	1*
Proliferation { Ducts Acini	1, 2	2, 3	1, 2	3	1	3	1		1		0, 1§	2
	2	2, 3	0, 1	3	0, 2	3	1		0		0, 1§	2
Secretion in ducts and acini	2	3	2, 1	3	0, 1	3	2		1		0, 2	2
Involut onary change	1	0	3	0	3, 1¶	0	1, 2		0		0	0

* Papillary cystadenoma occurred in a noncancerous mammary gland

† One animal showed an intraductal papilloma in a noncancerous mammary gland

‡ An adenomatous nodule was seen in the mammary gland of a 20 month old animal

§ Grade 1 was observed in 1 animal

|| Grade 2 was observed in 1 animal

¶ Grade 3 was observed in 2 animals

TABLE 2—*The Degrees of Proliferation, Secretion and Involutionary Changes Observed in the Mammary Glands of Castrate Male Mice*

Age at death, mo Interval since last injection, mo	Younger Age Group (Treated with Estrogen from the Age of 1 Month on)						Older Age Group (Treated with Estrogen from the Age of 4 Months on)					
	8 11		12 14		15+		8 11		12 14		15+	
	2 5		6-8		9+		0 2		3 5		6+	
	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer	No Cancer	Can cer
Cases	2	2*	4	3*	2	2†	1	3	2	2	8*	2‡
Proliferation { Ducts Acini	2	2, 3	2	2, 3§	2	2, 3	2	3	1	2	1	2
	2, 3	3	2, 3§	2, 3	1, 2	3	2	2, 3§	1, 2	3	1, 2¶	2
Secretion in ducts and acini	3	3	2	3	1, 2	2, 3	2	3	1	3	1	2, 3
Involutionary change	0, 1	0, 1	0, 1#	0	1, 2	0, 1	0	0	0	0	0, 1#	2¶, 0

* Intraductal papilloma was observed in 1 animal

† A microscopic carcinoma was observed in one grossly noninvolved mammary gland

‡ A microscopic carcinoma was observed in two grossly noninvolved mammary glands of the same animal

§ Grade 3 was observed in 1 animal

|| Grade 3 was observed in 2 animals

¶ Grade 2 was observed in 2 animals

Grade 1 was observed in 1 animal

the ductal epithelium showed foci of papillary projections. On the whole, there was little or no fibrosis, although in 2 of the 8 mice somewhat larger amounts of periacinous connective tissue were observed.

Animals in Which Breast Cancer Had Developed—In all 7 animals the non-cancerous breast tissue was in an advanced state of proliferation and showed

hypertrophy with abundant secretion in ducts and acini. The stimulation of growth was most pronounced in the animals 8 to 11 months old, it was somewhat less accentuated in the older mice. There was no evidence of a return to a resting state. In 1 mouse, 9½ months of age, diffuse adenosis was noted in all glands. Two other animals, 11 and 14 months old, respectively, showed marked stimulation of acinous growth in one noncancerous gland but less in the other two breasts. In a 19 month old mouse, two grossly noninvolved mammary glands had undergone early carcinomatous change.

The changes found in the various groups were graded and are summarized in tables 1 and 2.

A schematic tabulation has the disadvantage that certain variations of detail cannot be properly recorded. However, such a presentation facilitates a comparison of the main findings, particularly if a larger number of experimental groups is involved. Mammary growth processes, amount of secretion and involutionary changes were graded and included in the tables. The three grades of stimulation of growth used are essentially the same as those used by Loeb and Suntzeff³. Grade 0 designates the resting state.

Changes in the Ducts. Grade 1 indicates focal increase in number and size of the lining cells with an occasional mitotic figure, grade 2 is characterized by diffuse proliferation and hypertrophy of the epithelium associated with marked dilatation, elongation and budding of ducts, changes marked as grade 3 consist of pronounced proliferation and hypertrophy of the epithelium, more frequent mitotic figures, hyperchromasia of the nuclei and infolding of the epithelium into the ductal lumen.

Changes in the Acini. Grade 1 is characterized by focal increase in the number of acini, grade 2 indicates more widespread new formation of acini with mitotic proliferation and hypertrophy of the epithelium, whereas in grade 3 there were numerous closely packed hyperplastic and hypertrophic acini supported by thin strands of connective tissue.

Secretion. Grade 1 designates the presence of isolated shreds of eosinophilic material in the ductal lumen and small intracellular vacuoles, grade 2, thick globules of eosinophilic material in ducts and large secretory vacuoles in the lining cells, grade 3, occlusion of many enlarged ducts and acini by abundant eosinophilic secretions.

Involutionary Changes. Grade 1 indicates decrease in size and number of the acini with solution of the secretions and slight increase of the periacinous connective tissue, grade 2 designates collapse and fragmentation of acini with releasing of secretions into the supporting stroma, phagocytosis of secreted material and further increase of connective tissue, grade 3 is characterized by the presence of connective tissue arranged in a lobular fashion and rarely containing an atrophic epithelial cell. The latter condition may be spoken of as collapse fibrosis. The supporting connective tissue, which had increased during the period of stimulation of growth, remained preserved for longer periods than the epithelium, and it still appeared increased in amount when the epithelium had undergone regression. This increase was, however, relative rather than absolute, the connective tissue becoming conspicuous because of the involution of the glandular elements. At later stages of involution this fibrous tissue was in turn infiltrated by adipose tissue.

COMPARISON OF THE CHANGES IN NONCASTRATES OF THE
YOUNGER AND OLDER AGE GROUPS

Animals in Which No Breast Cancer Had Developed—On the whole, the mice of the younger age group showed more marked stimulation of mammary growth than those of the older age group. Only in 1 of the 8 animals of the latter group was there found a small conglomeration of acini (stimulation of grade 2). In the younger group 3 of the 7 mice showed grade 2 proliferation of the acini. The intensification of growth in the younger age group seems even more significant in view of the fact that animals of a given age in the older group were actually three months closer to the last injection than animals of corresponding age of the younger group. In other words, any involutionary changes that might counteract the effects of preceding stimulation of growth would have acted three months longer in the younger than in the older age group. Actually, in the younger series the processes of involution increased with increasing interval after the last injection, whereas in the older age group involutionary changes were less pronounced at all stages after discontinuation of the treatment. Therefore, the conclusion seems justified that, from the beginning on, the stimulation of growth was by far less accentuated in the older group than in the younger series.

Animals in Which Breast Cancer Had Developed—The growth stimulation of the noncancerous glands of the 1 animal of the older age group was less marked (grade 2) than that observed in the younger age group (grade 3).

COMPARISON OF THE CHANGES IN NONCASTRATES AND CASTRATES OF
THE YOUNGER AGE GROUP

Animals in Which No Breast Cancer Had Developed—At any given age the stimulation of ductal and particularly of acinous growth and secretion was more impressive in the castrates than in the animals with intact testicles. This became more obvious as the interval between the last injection and the death of the animal increased. In 5 of the 6 castrates over 12 months of age there was still active (grade 2 or 3) ductal and acinous growth and secretion, of the 5 noncastrate mice of corresponding age, only 1 showed grade 2 acinous stimulation, whereas in the remaining 4 animals a return to a resting or almost resting state had occurred. Thus, involution was more rapid and more complete in the noncastrates than in the castrates.

Animals in Which Breast Cancer Had Developed—There was no appreciable difference in the reaction of the mammary glands of castrates and noncastrates, both showing intense ductal and acinous stimulation. This applied to the general processes as well as to the more localized formation of benign intraductal papillae.

COMPARISON OF THE CHANGES IN CASTRATES AND NONCASTRATES OF
THE OLDER AGE GROUP

Animals in Which No Breast Cancer Had Developed—In the castrates, as a rule, epithelial growth, although not exceeding grade 2, was still more active than in the noncastrate animals. Of 8 noncastrate mice, only in 1 animal, 20 months old, was stimulation of acinous growth of grade 2 noted in one of the four mammary glands. Involutionary changes were delayed in the castrates as compared with the noncastrates, and in the latter such changes were seen only at early stages after cessation of the estrogenic treatment. The absence of these involutionary processes in noncastrate mice over 12 months of age is in agreement with the fact

that in this group the maximum stimulation had not exceeded grade 1 except in 1 mouse. Involutionary changes cannot be expected to be conspicuous in glands in which there had been only slight stimulation of growth.

Animals in Which Breast Cancer Had Developed—In the 1 noncastrate in which a mammary cancer had developed, conditions were essentially similar to those in the castrates bearing mammary gland carcinoma.

COMPARISON OF THE CHANGES IN CASTRATES OF THE YOUNGER AND OLDER AGE GROUPS

Animals in Which No Breast Cancer Had Developed—In mice of the younger age group the degree of stimulation of the breast tissue was rather uniform up to eight months after the last injection. Subsequently there was a slight decline of growth and correspondingly there was some increase in the involutionary changes. In the older age group the maximum stimulation was one degree lower than that in the younger age group. This difference may be, however, more significant than it appears, since again animals of the older age group were three months nearer to the last injection at the time of their death than those of identical age of the younger age group. Processes of involution were slight in the younger and practically absent in the older age group, in the latter, only 2 of the 8 mice over 15 months of age showed an involutionary change of grade 2.

Animals in Which Breast Cancer Had Developed—There was in the two age groups of castrates hardly any difference in the degree of stimulation of the grossly noncancerous mammary glands.

COMPARISON OF THE CHANGES IN THE BREASTS OF MICE BELONGING TO DIFFERENT AGE GROUPS BUT LIVING FOR A COMPARABLE PERIOD OF TIME AFTER THE LAST INJECTION

The interval which has elapsed since the last injection is of importance for the appraisal of the changes taking place in the mammary gland. During this time, either growth processes may increase, resulting in cancerous growth, or involutionary processes may set in and counteract the preceding preparatory stimulation of growth, thus preventing the transition from the preparatory to the cancerous phase and leading to a restoration of the resting state. In order to evaluate the effect of age on the course of growth after the cessation of the injections, it seemed necessary to supplement the foregoing tables by a comparison of animals of both age groups living for a comparable period after the last estrogenic injection. Animals of the older series 12 to 14 months of age should therefore be compared with mice of the younger series 8 to 11 months old, and animals of the older series 15 and more months of age should be compared with those of the younger series 12 to 14 months old.

Animals in Which No Breast Cancer Had Developed—Animals with Intact Testicles. Stimulation of mammary growth was distinctly less accentuated in the older than in the younger age group. Three to five months after the last injection the mice of the older group 12 to 14 months of age showed no acinous and only slight ductal proliferation, whereas acinous growth of grade 2 was present in the 8 to 11 month old animals of the younger age group. Involutionary changes were lacking in the animals of the older group, whereas they were present, though slight, in the younger series. As discussed in a foregoing section, this indicates that in the older group the stimulation of growth had been less pronounced than in the younger group from the beginning and that therefore less tissue could

undergo involution. A comparison of the findings in mice of the older age group 15 or more months of age and those of the younger age group 12 to 14 months of age—both killed six or more months after the last injection—led to similar results. In the younger age group there was more breast tissue present, and involutionary processes were more marked, than in the older age group.

Castrate Animals. In mice of the older age group 12 to 14 months old and living three to five months after cessation of the estrogenic treatment, ductal and acinous growth showed a stimulation of grade 1 or 2, in the mice of the younger age group 8 to 11 months old and living for three to five months after the last injection, the growth processes were those of grade 2 or 3. Correspondingly, most mice of the older group (6 of 8 animals) 15 or more months old and living six or more months after the last injection showed stimulation of mammary growth of grade 1, whereas in the 4 castrates of the younger age group 12 to 14 months old and living six and more months after the last injection the stimulation of growth was of grade 2 or 3. Involutionary changes in all groups of castrates reached a noticeable degree only at later stages after discontinuation of the estrogenic treatment.

Animals in Which Breast Cancer Had Developed.—The degree of growth stimulation in the grossly noninvolved mammary glands was about the same in the two age groups.

COMMENT

The results of the comparisons made in the preceding paragraphs may be summarized as follows:

Alpha estradiol benzoate injected from the age of 1 month intensified mammary growth of male mice of strain C3H with intact testicles. If administration of the estrogen was begun at 4 months of age, mammary growth was by far less stimulated than in the younger age group. This effect is in agreement with the different incidence of mammary carcinoma in these mice whose treatment began at different ages (15.8 per cent in the younger and 4 per cent in the older age group^{1b}). Moreover, in the noncastrates the mammary glands rapidly returned to a resting state after discontinuation of the injections. The difference in the responsiveness of the mammary glands of the two age groups might be due to the presence of the testicles, to an age factor or to both.

In male castrates of strain C3H the estrogen caused a more marked intensification of mammary growth than in the corresponding animals with intact testicles, irrespective of the age at which the injections were begun. Likewise, after discontinuation of the injections the involutionary processes proceeded more slowly in castrates than in noncastrates. The antagonistic effect of the testicular hormone (androgen) on estrogen-induced growth of the mammary gland⁴ could thus be confirmed.

⁴ Lacassagne, A., and Raynaud, A. *Compt rend Soc de Biol* **131** 186, 1939. Nathanson, I. T., and Andervont, H. B. *Proc Soc Exper Biol & Med* **40** 421, 1939. Jones, E. E. *Cancer Research* **1** 787, 1941. Heiman, J. *ibid* **4** 31, 1944. Gardner, W. U. *ibid.* **6** 493, 1946.

In castrate males of strain C3H treated from the age of 1 month on, the growth stimulation of the mammary gland reached a higher degree than in castrates receiving the estrogen from the age of 4 months on. An age factor independent of the testicle thus aids in determining the response of the mammary gland to estrogen not only as far as carcinogenesis is concerned but also as regards the progress of the preparatory period of growth. In castrates of both age groups, however, there were only slight and slowly progressing involutionary changes in the acini as the interval from the last injection to the death of the animal increased. The age factor seems, therefore, to play a minor role in the progress of involution of the mammary glands stimulated previously by the estrogen.

TABLE 3—*The Relative Significance of the Age Factor and the Testicular Hormone as Antagonists of Estrogenic Stimulation of the Mammary Gland*

Interval After Last Injection		Ratio of Acinous Growth in		Indicates Role of
I	2.5 mo 6.8 mo	Young Noncastrate	Old Noncastrate	Testicular Hormone and Age Factor
		2 ½	0 0	
IIa	2.5 mo 6.8 mo 9+ mo	Castrate Young	Noncastrate Young	} Testicular Hormone
		2½ 2¼ 1½	2 ½ ⅔	
		Castrate Old	Noncastrate Old	
IIb	0.2 mo 3.5 mo 6.8 mo	2 1½ 1¼	1 0 0	
		Young Castrate	Old Castrate	Age Factor
		2½ 2¼	1½ 1¼	

Table 3 illustrates the relative significance of the age factor and the testicular hormone as regards their inhibiting effects on estrogen-induced growth of the mammary gland. It represents a condensation of tables 1 and 2 with only the grades of acinous growth shown, for the sake of simplification. The figures were arrived at by adding the grades of acinous stimulation found in the individual animals and dividing the total by the number of animals in the respective groups. In view of the difficulty of expressing microscopic changes in numerical terms, the tabulation should be considered as an aid in discerning trends rather than as a basis for an exact statistical analysis.

The most powerful inhibition of mammary growth (horizontal column I) was exerted by the combined action of both the testicle and the age factor, the latter presumably being due to constitutional factors inherent in the breast tissue itself as pointed out by Loeb¹. The part played by the testicular hormone in the inhibition of estrogen-induced mammary growth is indicated in horizontal columns IIa and IIb.

At the time of the maximum stimulation, two to five months after the last injection, there was but one-half degree difference between castrates and noncastrates of the younger group (column IIa) and one full degree between castrates and noncastrates of the older age group (column IIb). The inhibiting effect of the testicular hormone appears, therefore, to be comparatively slight. However, if the changes of acinous growth seen with increasing interval from the last injection (vertical column in horizontal column IIa and IIb) are considered, the marked influence of the male sex hormone (androgen) becomes more evident. The return to the resting state was accelerated in the presence of the testicle, suggesting that the latter is largely responsible for the progress of involutionary changes in the mammary glands of those mice in which no cancer had developed. The inhibitory effect exerted on mammary growth by the age factor (horizontal column III) approximates that exerted by the testicle during the early stages following treatment. However, the age factor failed to influence significantly the progress of involutionary processes.

In the comparatively few noncastrate animals in which mammary cancer developed, the mammary gland tissue must have been sufficiently responsive or the stimulus acting on the acini must have been powerful enough to overcome the restraining influence of both the testicular androgen and the age changes taking place in the mammary gland tissue. This diminution of resistance was confirmed by the microscopic appearance of the noncancerous mammary glands. In the latter the growth processes were distinctly stimulated, while involutionary changes were absent or insignificant.

SUMMARY

In castrate and noncastrate male mice of strain C3H treated with an estrogen the growth of the mammary gland was stimulated even in those animals in which mammary cancer did not develop. The intensification of growth processes paralleled closely the incidence of breast cancer in castrate and noncastrate mice treated with the estrogen. In castrates of a given age the mammary growth was more enhanced than in noncastrates of a corresponding age, and in castrates as well as in noncastrates receiving the estrogen during earlier periods of life the growth stimulation exceeded that seen in animals treated similarly at later ages. Processes of involution proceeded more rapidly in the presence of the testicles irrespective of the age of the animal at the time of the treatment.

Thus at least two factors are involved in the inhibition of estrogen-induced growth of the male breast: (1) the male sex hormone (androgen) which inhibits mammary growth during estrogenic treat-

ment and promotes involutionary processes after discontinuation of the treatment, (2) an age factor inherent in the aging breast tissue and opposing the stimulation of growth caused by the estrogen, in the involution of the stimulated mammary gland the role of this age factor is less conspicuous than that of the testicle

The noncancerous glands of the mice in which breast cancer had developed showed a high degree of stimulation throughout. These findings, in conjunction with those obtained in the mammary glands of noncancerous mice, suggest that factors which promote or inhibit cancerous growth also intensify or oppose those phases of growth that precede cancer formation.

GASTRIC PARAGANGLIOMA WITH ULCERATION

Report of a Case

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AND

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ROCHESTER, N Y

THE PURPOSE of this paper is to report a paraganglioma occurring in the wall of the stomach, to review the literature and to remark on the existing lack of agreement in classification and terminology concerning this type of tumor

Chromaffinoma, or paraganglioma, was first described, according to Wahl,¹ in 1883 by Weichselbaum, and similar tumors were recorded by Frankel in 1886 and by Perley in 1890, according to Lewis and Geschickter.² Reid³ cited Berdez as having reported a case in 1892. Kohn is credited with having published the first description of the chromaffin system in 1902. He included the carotid body, the gland at the aortic bifurcation, the medulla of the adrenal gland and the vertebral sympathetic ganglions. Subsequent authors⁴ made additions or deletions in this original definition of the chromaffin system. Recent papers⁵ have tended to separate the carotid body tumor and the carcinoid, or argentaffinoma, from the paraganglioma.

The embryology of the paraganglion cells, which give rise to these tumors, deserves a word. The neural crest gives rise to immature cells which migrate along the sympathetic nervous system. Some come to rest in the form of small masses in concavities on the dorsal surfaces of the sympathetic ganglions, hence the term "paraganglions." Before these cells mature into the adult chromaffin cells, other primordial sympathetic nerve cells migrate to the adrenal medulla, the abdominal sympathetic plexuses, the organs of Zuckerkandl and elsewhere in the autonomic nervous system. These sympathetic cells have been described in the thorax, the retroperitoneal tissues, the renal hilus, sacrococcygeal

From the Department of Pathology, Genesee Hospital

1 Wahl, H R J M Research **30** 205, 1914

2 Lewis, D, and Geschickter, C F Arch Surg **28** 16, 1934

3 Reid, M R Ann Surg **88** 516, 1928

4 Guild, S R Anat Rec **79** 28, 1941 Lewis and Geschickter²

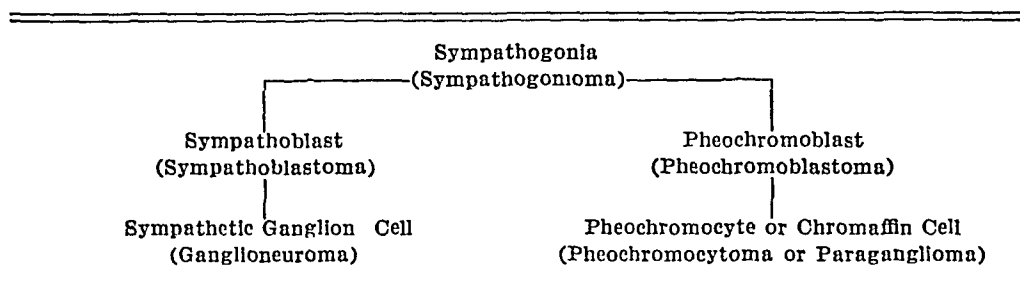
5 (a) Brines, A O, and Jennings, E R Am J Path **24** 1167, 1948

(b) Waaler, E Acta med Scandinav **123** 1, 1945 (c) Philips, B Arch Path **30** 916, 1940

tissue, the celiac ganglion, the breast, the arm, the tibia and the upper part of the sternum⁶ Bielschowsky⁷ stated in his discussion of the embryogenesis of the sympathetic ganglions that the primitive neurocytes or sympathogonia are the mother cells not only of the sympathoblasts and sympathetic ganglion cells but also of the chromaffin cells The dichotomous division of the "family tree" as presented by Bielschowsky is shown in table 1 The corresponding tumor is inserted with its cell of origin

Bielschowsky added that the genealogy of the sympathogonia is not sharply defined inasmuch as certain tumors reveal elements of both neuroblastic and pheochromic characteristics Wahl¹ in 1914 recognized this fact, stating that examples of one pure type are rare He reported a case illustrating three anatomically distinct types Later, in 1943, in conjunction with Robinson, Wahl⁸ reported a case of neuroblastoma of the mediastinum with a pheochromoblastomatous ele-

TABLE 1—*Dichotomous Division of the "Family Tree" of the Sympathogonia According to Bielschowsky*⁷



ment The latter tumor is described as showing neurocytes, ganglion cells, nerve fibrils, pheochromoblasts and pheochromocytes In 1939 Potter and Parrish⁹ reported a 2 month premature infant which at autopsy revealed a tumor made up of sympathogonia, sympathoblasts and ganglion cells An anatomically separate schwannoma was also found in this infant To complete the consideration of the vagaries of which these tumors of sympathetic system origin are capable, the case reported by Cushing and Wolbach¹⁰ in 1927 should be mentioned, in which there was transformation of a cancerous paravertebral sympathoblastoma into a benign ganglioneuroma

Chromaffin tumors are rare According to Brines and Jennings,^{5a} approximately 210 have been reported The terms "paraganglioma,"

6 Sailer, S Am J Path **19** 101, 1943

7 Bielschowsky, M, in Penfield, W Cytology and Cellular Pathology of the Nervous System, New York, Paul B Hoeber, Inc, 1932, p 1083

8 Wahl, H R, and Robinson, D Arch Path **35** 571, 1943

9 Potter, E L, and Parrish, J M Am J Path **15**:652, 1939

10 Cushing, H, and Wolbach, S B Am J Path **3** 203, 1927

"chromaffin tumor" and "pheochromocytoma" are found to be used interchangeably in the literature, various authors employing the same term to denote histologically different tumors. Henceforth, we shall speak of paraganglioma as Pick¹¹ first defined the tumor in 1912, those chromaffin tumors occurring without the adrenal gland, in contrast to those which occur within the adrenal gland, which are designated pheochromocytoma. Waaler^{5b} collected all the cases of paraganglioma in the literature up to 1945. He cited Gormsen's review with 20 cases of paraganglioma and added 18 of his own. On reviewing the literature since 1945 one notes that approximately 8 additional cases, including our own, of extra-adrenal paraganglioma have been reported.

REPORT OF CASE

A 38 year old white man, employed as a commercial chauffeur, was admitted to the Genesee Hospital, Rochester, N. Y., Nov 8, 1948, with the chief complaint of heaviness and pain in the stomach of two years' duration. The patient stated that two years previously he began to note postprandial heaviness and discomfort in his stomach, which often lasted for several hours. Several months later, the gastric distress, described as a dull pain in the pit of the stomach, became preprandial, and was relieved by food. Approximately one year before admission, the pain became postprandial again, appearing one-half to one hour after meals. This dull postprandial pain, together with a continually increasing feeling of gastric heaviness, persisted. The patient recalled one large rectal hemorrhage and that subsequently he had observed dark stools for a period of two months prior to admission. During the course of his illness, the patient had been treated by four or five different physicians for peptic ulcer and had found that milk relieved his pain temporarily. He suffered a 20 pound (9 Kg.) loss of weight during the year prior to admission, together with occasional weakness and lethargy.

At the time of admission the temperature was 99 F, the pulse rate 76, the respiratory rate 20 and the blood pressure 130 systolic and 80 diastolic. There was slight epigastric tenderness to deep palpation. No masses were felt. The patient's hemoglobin was 12.1 Gm per hundred cubic centimeters, the red blood cell count was 4,200,000. The radiologist Dr G. J. Baron reported on a gastrointestinal series made Nov 8, 1948 as follows: "No abnormalities are noted in the esophagus. The stomach in general shows a good outline with perhaps slight hypertrophy of the rugae. Peristalsis is quite active in the proximal portions of the stomach. In the pyloric antrum there is a large, smooth, quite well defined filling defect with a broad base, which appears to arise from the lesser curvature. The outline of this defect is not particularly irregular, and rugal markings can be seen in the filled portion of the stomach in this area. There does not appear to be significant obstruction, barium sulfate passing freely into the duodenal bulb, which shows normal outline and activity. No abnormality is noted in the duodenal loop or the upper part of the small bowel." The interpretation of the finding was that it might be a benign polyp but that more likely it was a cancer, because of the broad base. Figure 1A is a reproduction of the roentgenogram of the stomach.

11 Pick, L. *Berl klin Wchnschr* 49 16, 1912

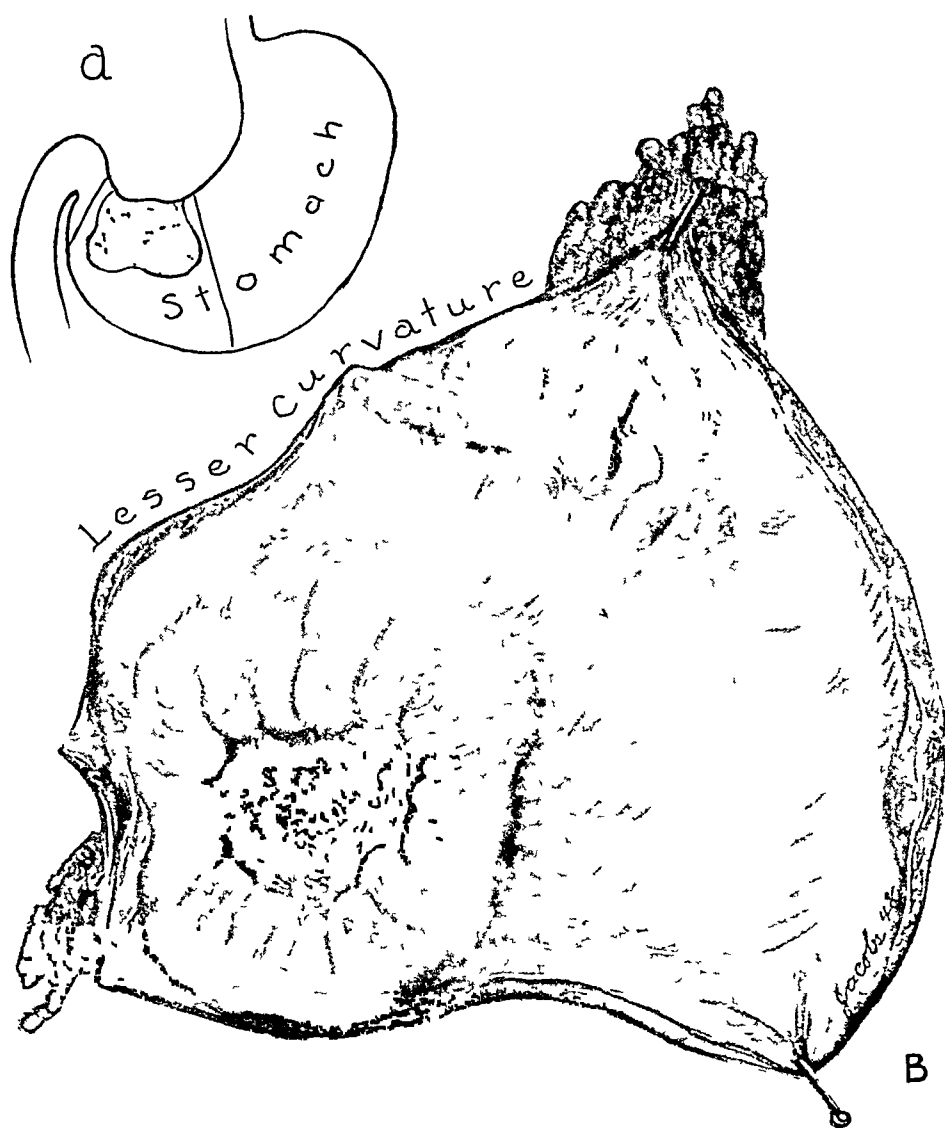


Fig 1—*A*, roentgenogram of the stomach showing the prepyloric defect *B*, drawing of the gross specimen showing the ulcer and indicating (*a*) its prepyloric location

On November 9 the patient underwent an exploratory laparotomy (Dr T B Jones) The exploration gave negative results except for some rather large succulent lymph nodes extending along the aorta and great vessels The local lesion was found to involve the prepyloric region of the stomach The operator described an irregular lobulated elastic tumor invading the superior and posterior aspect of the stomach It was freely movable and unattached to surrounding structures The tumor itself could be moved over the underlying serosal layers No enlarged lymph nodes were noted in the immediate vicinity of the tumor Resection was decided on and a Billroth anastomosis was done The postoperative course was uneventful At the time of writing the patient is asymptomatic and reports a 15 lb (6.5 Kg) gain in weight seven months after operation

The specimen consisted of a 14 by 7 cm circular segment of stomach On its posterior aspect the serosa showed a firm, raised, rounded subserosal mass measuring 1.5 by 1 cm The opened specimen revealed a raised, firm, smooth, oval, sessile excrescence measuring 5 by 3 by 2.5 cm on the lesser curvature 2 cm from the distal resection margin The center of this mass showed a punched-out mucosal ulcer crater measuring 1.5 cm in diameter and 0.8 cm in depth The edges of the ulcer were depressed, and the gastric mucosa was pearl gray (figure 1 B) On section the mass revealed pale brown tumor tissue with concentric areas of shiny gray mucoid-like tissue interrupting the structure and extending from submucosa to serosa Some of these areas gave the impression of small onions in cross section, with circular laminae and easily shelled out central portion The tumor also appeared in some areas to dissect through the muscle layers in thin sheets No evidence of encapsulation was found

Microscopic sections showed gastric mucosa, revealing in one area ulceration with granulation tissue, chronic inflammatory cells, hemorrhage, edema and necrosis, filling in the base of the ulcer Elsewhere throughout the submucosa and extending through the muscularis to the serosa were sheets of tumor cells, in some areas forming rosettes and in other areas nests and cords (fig 2 A) The cells generally had small round or oval nuclei with discrete nucleoli The cell cytoplasm was acidophilic and vacuolated There were a few scattered cells containing large, pleomorphic, hyperchromatic nuclei, some containing multiple nuclei (fig 2 B) Wilder's stain showed an argyrophilic stroma supporting the tumor cells, but no evidence of an intracellular reticulum was demonstrated Fat stains (sudan IV) showed no intracellular fat Many of the tumor cells presented faint brown intracytoplasmic stippling after being fixed in chromate solution Throughout the tumor were spaces containing an acidophilic colloid-like material showing some vacuolation (fig 2 C) Scattered lymphocytes and polymorphonuclear leukocytes were present

Sections of regional lymph nodes showed no atypical cells

In reviewing all nonepithelial tumors of the stomach reported in the literature we found 1 case similar to our own This was reported by Bindsvle¹² in 1941, whose report has been translated for us by Dr Einar Lie The case is described as one of chromaffin tumor of the lesser omentum invading the stomach wall in a 15 year old youth The presenting symptoms were severe anemia and melena The patient was given seven transfusions of whole blood and then submitted to a surgical exploration of the abdomen Splenectomy and extirpation of the tumor followed In the lesser omentum and stomach was a 5 by 4 by 4 cm firm tumor The tumor lay in the stomach wall, penetrating the mucosa and forming a 1 by 1 cm funnel-shaped ulcer The postoperative course was uneventful The specimen

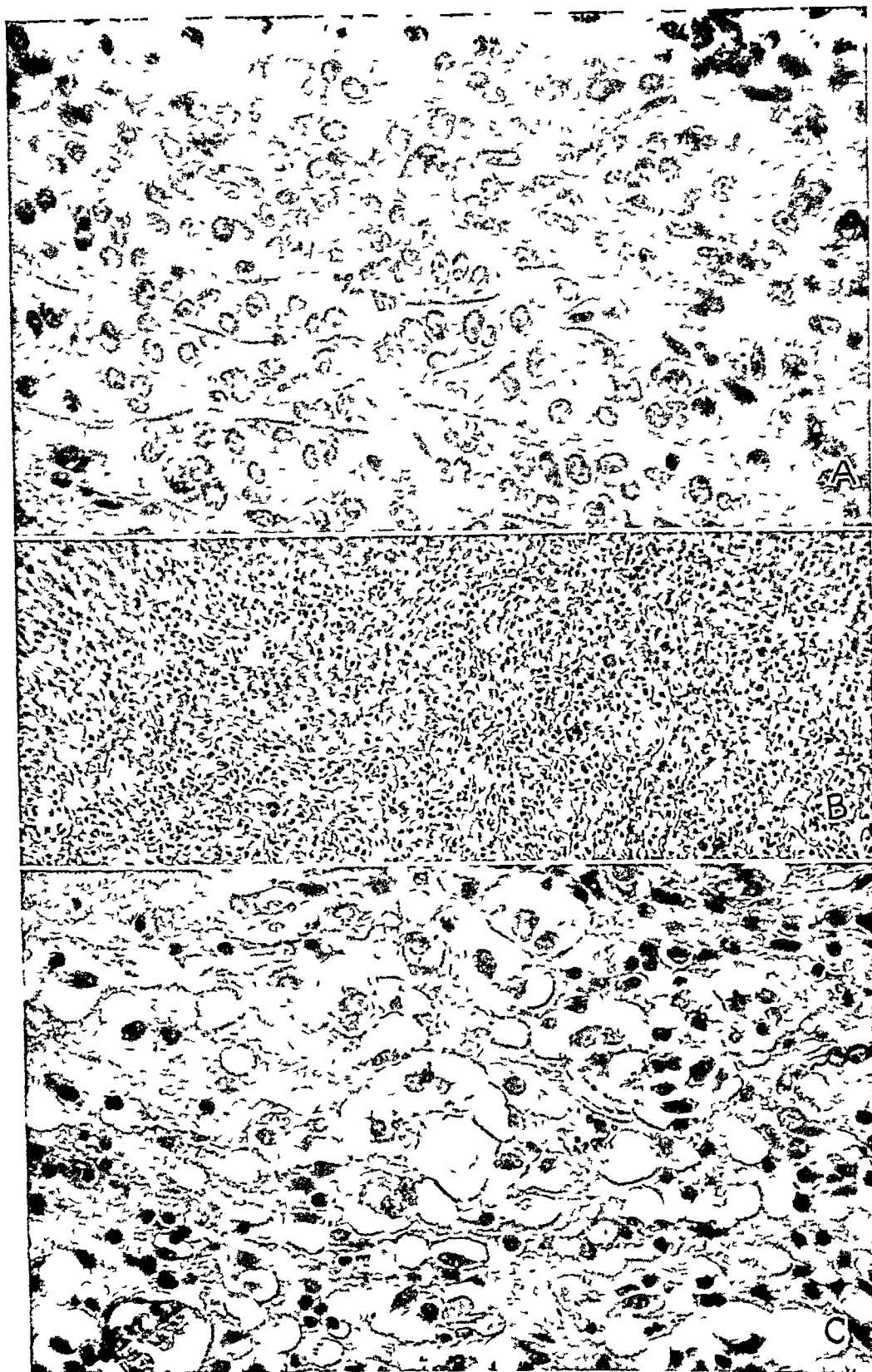


Fig 2—*A*, nests and cords of cells in the wall of the stomach *B*, cellular structure of the tumor *C*, colloid-like material with vacuolation

showed a shell of tumor tissue around a central hemorrhagic mass. The tumor cells formed strands and rosettes with some alveolar patterns. The cells were rich in protoplasm with indefinite cell boundaries and round or oval nuclei. Centrally the tissue was necrotic, with lymphocytic infiltrations and old blood pigment. The cells penetrated through the submucosa into the muscularis mucosae. In the submucosa the indefinite cell borders gave the tumor an aspect of cancerous change. The microscopic diagnosis was paraganglioma of the stomach. The spleen showed fibrosis, and a diagnosis of Banti's disease was made.

It is interesting to note that ulceration of the tumor mass associated with hemorrhage was present in both Bindslev's and our own case. Dupuy¹³ in 1925 reported a case in which hypertrophy of Auerbach's plexus was found beneath a gastric ulcer. He suggested that the ulcer might have some causal relationship. Masson¹⁴ in 1921 attempted to establish a relationship between proliferation of nerve tissue in the wall of the stomach and gastric ulcers. Canney¹⁵ in 1948 stated that

TABLE 2—*Intra-Abdominal Neoplasms of Neurogenic Origins*
(After Ransom and Kay¹⁶)

1	Nerve sheath tumors
A	Benign
1	Neurilemmoma (schwannoma, perineural fibroblastoma, etc)
2	Neurofibroma
3	Cirsoid or plexiform neurofibroma
4	Ganglionated neurofibroma
B	Cancerous
1	Neurogenic sarcoma
2	Neuroblastic tumors of sympathetic system
1	Sympatheticoblastoma
2	Paraganglioma
3	Ganglioneuroma

there is little evidence relating proliferated nerve tissue to true benign neurogenic growths.

Recent articles on neurogenic tumors of the stomach follow a classification employed by Ransom and Kay¹⁶ (table 2).

Ransom and Kay¹⁶ in 1940 reported 18 cases of intra-abdominal neurogenic tumors made up of neurilemmoma, neurogenic sarcoma, neurofibroma and ganglionated neurofibroma, and Canney¹⁵ in 1948 reported a case of neurilemmoma and a case of neurofibroma occurring in the stomach. Lockwood¹⁷ in 1932 reported a case of noncancerous neuroblastoma of the stomach. Pitts¹⁸ in 1947 described a case of ganglioneuroma of the stomach. In 1934 and 1937 a sympatheticoblas-

13 Dupuy, M. *Internat Clin* 4 164, 1925

14 Masson, P. *Compt rend Acad d sc* 173 262, 1921

15 Canney, R. L. *Brit J Surg* 36 139, 1948

16 Ransom, H. K., and Kay, E. B. *Ann Surg* 112 700, 1940

17 Lockwood, B. C. *J A M A* 98 969, 1932

18 Pitts, H. H., and Hill, J. E. *Canad M A J* 56 537, 1947

toma was reported occurring in the stomach wall in 2 cases by Cieza Rodríguez and Bianchi¹⁹ The two paragangliomas cited in this paper may now be added to complete the classification as concerns gastric neoplasms

COMMENT

With the reporting of this case of gastric paraganglioma and the presenting of Bindslev's case in English, attention is directed toward this rare tumor of the stomach and the associated gastric ulcer These 2 cases provide evidence for the occurrence of the heretofore postulated paraganglioma of the stomach, thereby completing case reports for the classification of Ransom and Kay¹⁸ as regards gastric tumors

Opportunity is also afforded for some specific comment on the nomenclature of tumors of the chromaffin system Considerable confusion exists in regard to this group of neoplasms, because classifications by and large rest solely on the morphologic aspects of the tumor cells and do not consider the physiologic possibilities of the new growth Thus we would agree with Pick¹¹ and with Belt and Powell,²⁰ reserving the term "paraganglioma" for extra-adrenal tumors arising from cells of the chromaffin system, with the additional qualification that these extra-adrenal tumors exhibit no hormonal or other specific physiologic activity Tumors of the adrenal gland, while arising from a similar cell type, are not paragangliomatous in the true sense of the term and should not be classed as paraganglioma Tumors occurring in extra-adrenal situations which give rise to various clinical symptoms related to hormonal production and secretion should be excluded from a paragangliomatous designation, since the cells, while apparently morphologically similar, obviously have other qualities which make them quite different The biologic behavior of neoplasms is an important factor and must receive consideration together with the histologic information gleaned from microscopic analysis

SUMMARY

A case of paraganglioma of the wall of the stomach associated with mucosal ulceration and hemorrhage is reported

A similar case of gastric paraganglioma associated with mucosal ulceration, reported by Bindslev in 1941, is reviewed and summarized in English

It is suggested that the term "paraganglioma" be reserved for extra-adrenal tumors arising from cells of the chromaffin system and exhibiting no physiologic activity

19 Cieza Rodríguez, M., and Bianchi, A. E. *Bol y trab de la Soc de cir de Buenos Aires* **18** 1225, 1934 Bianchi, A. E., and Cieza Rodríguez, M. *Novena reunion Soc argent de pat reg* **2** 1018, 1937

20 Belt, A. E., and Powell, T. O. *Surg, Gynec & Obst* **59** 9, 1934

NORMAL OCCURRENCE OF HISTOLOGICALLY DEMONSTRABLE FAT IN THE LIVER OF THE NEWBORN INFANT

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DURING the routine microscopic examination of sections of liver from newborn and stillborn infants on whom autopsies were performed in Sinai Hospital, Baltimore, it was observed that fat occurred in the form of vacuoles in the parenchymal cells of the liver with much more frequency than one might expect on the basis of the associated pathologic processes. It was also observed that there appeared to be a distinct correlation between the amount of fat and the maturity or the degree of prematurity of the infant. Since "fatty change," "fatty infiltration of the liver" and other such diagnoses dependent on the histologic observation of fat in the liver carry a distinctly pathologic connotation, an attempt was made to determine whether or not the fat detected in the livers of newborn and stillborn infants by various staining methods was present normally and whether its presence could be correlated with body weight as an indication of maturity. No record of a similar study could be found in the literature.

MATERIALS AND METHODS

Thirty-one infants, representing all the newborn and stillborn infants subjected to postmortem examination in this hospital between Aug. 18, 1948 and May 8, 1949 whose period of survival was fourteen days or less, were used in this study with the exception of those classified as "foetus sanguinolentus." Portions of liver taken at autopsy were fixed in 4 per cent formaldehyde solution for forty-eight hours. Frozen sections were cut between 15 and 20 microns in thickness. They were stained a few seconds in Harris' hematoxylin and then stained for fat with sudan IV (modification of Herxheimer's solution¹)². Occasionally

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1 The staining solution was made up as follows

Scarlet red	15 Gm
Alcohol 70 per cent	100 cc
Acetone, chemically pure	20 cc

Sections were stained with Harris' hematoxylin for thirty seconds and then transferred to this solution for five minutes. They were then rinsed in 1:1 alcohol-acetone solution and mounted in Apathy's solution.

2 Cowdry, E. V. *Microscopic Technique in Biology and Medicine*, Baltimore, Williams & Wilkins, 1943

a 1 per cent solution of osmic acid was used, but this did not prove to be as satisfactory. The staining was at times capricious. Often several sections were stained before a particular specimen was accepted as showing or not showing fat. In most instances the presence of stainable fat could be predicted from the appearance of the permanent sections stained by the hematoxylin and eosin technic. In such sections, depending on the amount of fat present, numerous vacuoles could be seen within the liver cells. These vacuoles varied considerably in size, some being small, while others filled almost entire cells, giving them a signet ring appearance. The sudan IV-stained frozen section usually reflected the appearance of the permanent section stained with hematoxylin and eosin.³ Where the vacuoles were small in the permanent section, the sudanophilic droplets were finely dispersed in the frozen section. Where the majority of the liver cells were replaced by large vacuoles, the sudan IV-stained section would show large orange-red globules. In 1 instance, although the hematoxylin-eosin section had numerous vacuoles within the liver cells, the sudan IV stain revealed none. This case is classified as negative in the series. In all other instances where vacuoles could be seen on hematoxylin and eosin staining, fat could be demonstrated with the sudan IV stain.

Sections were mounted in Apáthy's solution and retained for future study. Within the time limits of the study no sections were observed to fade or otherwise lose their characteristic appearance. The amount of fat present was graded 0 to +++++ as follows:

- 0—an occasional stained droplet or none at all
- +—minimal. Staining was limited to sudanophilic droplets in an occasional liver cell in most of the lobules
- ++—moderate. Sudanophilic droplets were present in several liver cells in each lobule
- +++—marked. Sudanophilic droplets were present in approximately half of the liver cells in each lobule
- ++++—very marked. Almost all cells observed contained sudanophilic droplets

The maturity or prematurity of the infant was estimated most acceptably by the birth weight. Infants weighing 2,500 Gm and over were considered at term, those weighing less were considered premature. Four weighed less than 1,500 Gm. An attempt to classify the infants with respect to actual and expected dates of delivery proved unsatisfactory because of the errors inherent in calculations of the dates of confinement.

RESULTS

Of the 11 infants weighing 2,500 Gm or more, 10 had fat in the liver in "marked" to "very marked" amounts (+++ to +++++). The exception was a 2,800 Gm stillborn infant whose liver showed only moderate amounts of fat (++) in the 1,500 to 2,500 Gm group, consisting of infants considered to be premature, only 5 of the 16 had more than a minimal amount of fat in the liver. Three of these weighed between 2,400 and 2,500 Gm and could thus be consid-

3 It is realized that estimates of fat content based on the staining of fat droplets in frozen sections by the sudan IV method are gross approximations. Only neutral fats are demonstrated by sudan IV. Estimates of other lipid substances require careful chemical analysis and are beyond the scope of this paper. It is hoped that such studies will be undertaken to corroborate the findings presented here.

ered reasonably close to term. The remaining 11 infants in this group and the 4 infants weighing less than 1,500 Gm had little or no fat in their livers, the amounts being classified 0 to + (table 1). No correlation could be shown

TABLE 1—*Comparison of Infant Size as Indicated by Weight with Amount of Fat Observed in Liver*

Body Weight, Gm	Amount of Fat Observed *	Body Weight, Gm	Amount of Fat Observed *
3,810	++++	2,245	0†
3,407	++++	2,240	+
3,114	+++	2,125	+++
3,070	++++	2,104	0
3,015	+++	2,060	0
3,005	++++	2,045	++
3,000	++++	2,000	0
2,940	+++	1,820	0
2,800	++	1,635	+
2,790	++++	1,561	0
2,715‡	+++	1,525	0
2,497	++	1,250	0
2,460	+++	1,108	+
2,425	++++	815	+
2,320	0	550	0
2,300	+++		

* 0—none
+—minimal
++—moderate
+++—marked
++++—very marked

† The hematoxylin and eosin stain revealed many vacuoles, but these would not stain with sudan IV

‡ Weights of 2,500 Gm and over were considered full term, weights under 2,500 Gm were considered premature

TABLE 2—*Distribution of Survival Times in Comparison with Fat Observed in Liver*

Survival Time	0	+	++	+++	++++	Total
Stillborn	2		1			3
Less than 24 hr	5	2	1	2	1	11
24-48 hr	1	1		1	3	6
48-72 hr	1				1	2
72-96 hr				2		2
96 hr and over	2	1		2	2	7
						31

TABLE 3—*Associated Pathological Conditions†*

Congenital heart disease	9
Subarachnoid hemorrhage and hemorrhage into the falx	9
Congenital abnormalities of the gastrointestinal tract	5
Bronchopneumonia	5
Congenital hypoplasia of the kidneys	2
Hemopericardium, traumatic (result of intracardiac puncture)	1
Erythroblastosis fetalis	2
Prematurity ‡	8

* More than one diagnosis is listed for each case

† This was the primary diagnosis in instances in which no other more definitive morbid condition could be found

between the amount of fat in the liver and the survival time (table 2). There was no definite correlation with the associated pathologic changes (table 3).

COMMENT

This study shows that the presence of fat in the liver of newborn, full term infants is probably normal. Heretofore, it has usually been considered abnormal and has given rise to the diagnoses of "fatty change of the liver" and "fatty infiltration of the liver." Often the fat has been explained on the basis of anoxemia, but this can scarcely be expected to produce such an extensive distribution of fat within the short survival time of some of the infants studied. Of interest also is the fact that the more premature the infant the less the fat that can be demonstrated in its liver. The following arguments are offered in an attempt to show that histologically demonstrable fat in the liver is part of the normal physiologic and metabolic background of the newborn infant.

Boyd and Wilson,⁴ calculating the concentrations of lipid materials in the cord blood of human subjects, were able to show that umbilical vein blood leaving the placenta and supplying the fetus contained higher concentrations of certain lipid materials than umbilical artery blood returning to the placenta. They concluded that certain lipid substances are added to umbilical blood by the placenta and are removed or absorbed by the fetus. While studies are not available showing the rate at which fats are stored in the human fetus, Imrie and Graham,⁵ studying the fat content of the embryonic guinea pig liver, showed quite definitely that fat accumulates in the livers of guinea pigs during the latter part of gestation. No evidence of this accumulation was found until the embryos attained a weight of about 40 Gm. However, as their weights increased above this value, the increase in fat was quite impressive. The rapid disappearance of the fat after birth "suggests that it (fat) is material required by the young animal after its communication with the maternal circulation has ceased."

Many interesting studies have been made in an attempt to calculate the relative amounts of fat and carbohydrate metabolized by the newborn infant. Needham⁶ stated that in utero the respiratory quotient is close to 1.0, indicating that carbohydrate forms almost the only source of energy. Benedict and Talbot⁷ found the respiratory quotient in the neighborhood of 0.90 shortly after birth, reflecting the utilization of the stored glycogen available at birth. The amount of stored carbohydrate is apparently limited, however, and over the subsequent seventy-two

4 Boyd, E. M., and Wilson, K. M. *J. Clin. Investigation* **14** 7, 1935.

5 Imrie, C. G., and Graham, S. G. *J. Biol. Chem.* **44** 243, 1920.

6 Needham, J. *Chemical Embryology*, New York, The Macmillan Company, 1931, vol. 2.

7 Benedict, F. G., and Talbot, F. B. *The Physiology of the Newborn Infant. Character and Amount of Katabolism*, no. 233, Washington, Carnegie Institute, 1915.

hours the average respiratory quotient tends to fall, reaching its lowest value of 0.73 on the third day. This would correspond to a metabolic mixture of approximately 90 per cent fat. By the end of the first week the average respiratory quotient rises to about 0.80 and metabolic mixtures consisting of approximately one-third carbohydrate and two-thirds fat are being used. Once milk can be utilized in adequate amounts, the utilization of carbohydrate increases. These results indicate the importance of fat in the metabolism of the newborn infant, perhaps best summarized in this statement by Smith⁸

The fetus probably uses little fat for its heat production but stores a considerable amount against later emergencies, how this is brought across the placenta is not known. Once the newborn infant has fairly well depleted its available glycogen stores (which is usually within a few hours after birth) it begins to depend largely upon reserves of fat for energy. During this process there is a greatly increased transport of fat substances in the blood, an increase not solely due to the newly introduced element of absorption from the alimentary tract.

Two statements, then, can be made with regard to the normal presence of considerable quantities of histologically demonstrable fat in the liver of the newborn, full term infant.

- 1 In the interval between the rapid exhaustion of available carbohydrate stores and the assimilation of adequate dietary carbohydrate the fat stores in the full term infant provide a suitable, plentiful and potent source of energy.

- 2 Ample opportunity for such storing of lipid materials in the liver occurs in utero through placental transport.

The noticeable lack of fat stores in the liver of a premature infant is probably evidence that hepatic fat storage is a physiologic preparation for birth. From a study of the estimated dates of confinement of the mothers of infants in this series there is every indication that the storing of fat in the liver apparently begins in earnest during the last month of intrauterine life.

SUMMARY

Fat which can be histologically demonstrated by certain staining methods in the livers of newborn, full term infants is probably a normal finding.

By the use of the same methods it has been shown that premature infants do not have appreciable quantities of fat in their livers.

A possible physiologic explanation is offered for the normal presence of histologically demonstrable fat in the liver of the newborn infant at term and its absence in the premature infant.

⁸ Smith C. A. *The Physiology of the Newborn Infant*, Springfield, Ill., Charles C. Thomas, Publisher, 1946.

DEGENERATIVE RENAL LESIONS INDUCED BY PROLONGED CHOLINE DEFICIENCY

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AND

H P RUSCH, M D

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ALTHOUGH most studies on choline deficiency have been concerned with changes in the liver, a number of investigators have also observed the effect of this deficiency on the kidney. Hemorrhagic lesions of the kidneys of rats have been described repeatedly¹ since the initial observations of Griffith and Wade². In acute (less than six months) choline deficiency Gyorgy and Goldblatt^{1c} observed that the initial lesions were in the tubules, with glomeruli affected only in the terminal stages. Engel and Salmon^{1b} observed minimal thickening of the renal capsule with some connective tissue proliferation in the healing phase in rats subjected to acute choline deficiency. Christensen observed a rather remarkable degree of healing four to sixteen months after acute periods of choline deficiency^{1a}. In the healing phase he observed wedge-shaped areas of cortical atrophy, calcified tubular remnants and disappearance of eosin casts with persistence of dilated atrophic tubules.

Hartroft and Best³ succeeded in producing hypertension and renal changes in some of their rats when choline-deficient diets were maintained for periods longer than six months. After comprehensive study of the pathogenesis of the acute "hemorrhagic renal syndrome," Hartroft pointed out that the sudanophilic material accumulating in the proximal convoluted tubules was related to the subsequent cortical capil-

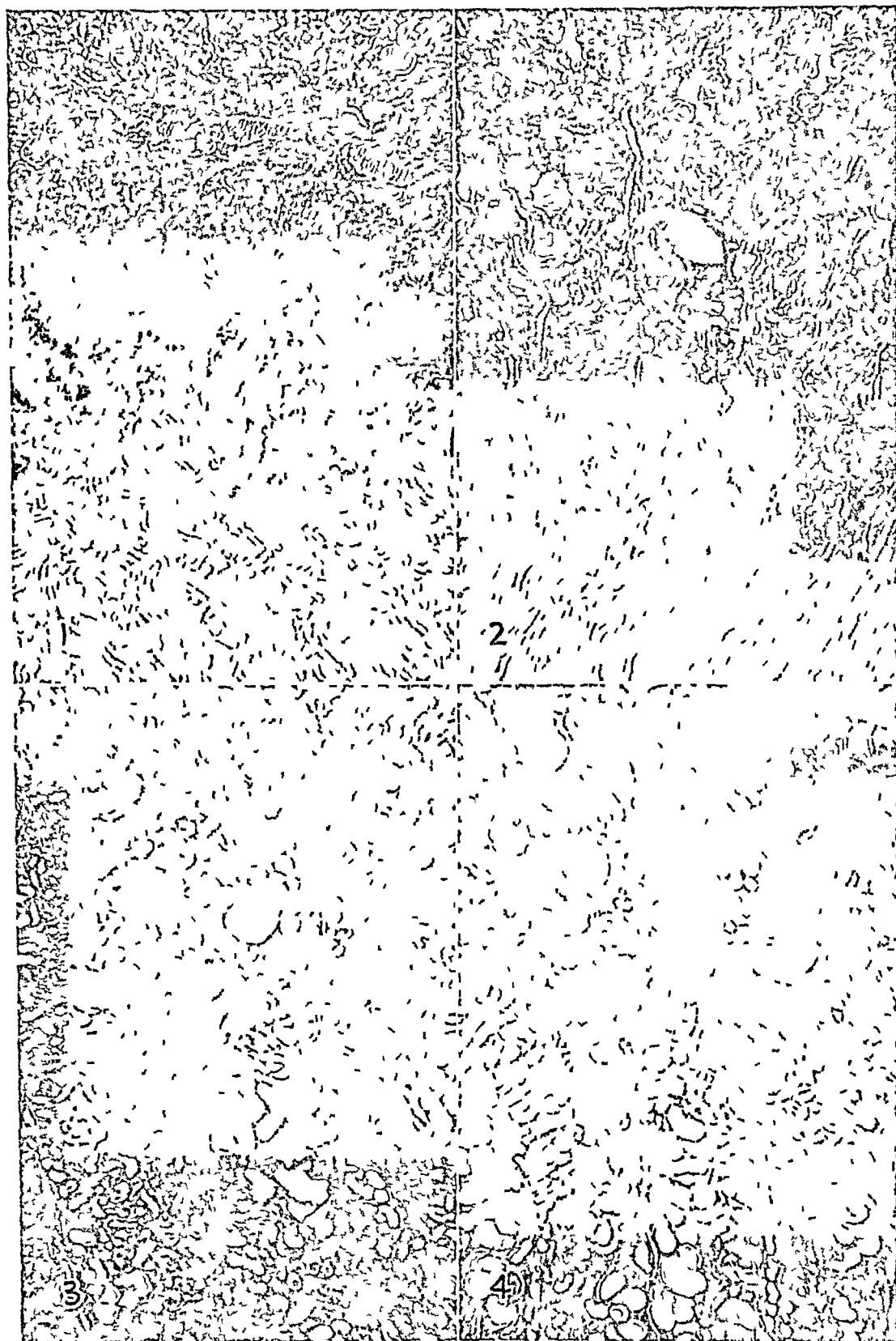
This investigation was aided by the Jonathan Bowman Fund for Cancer Research.

From the Department of Pathology and McArdle Memorial Laboratory, University of Wisconsin Medical School.

1 (a) Christensen, K. *Arch Path* **341** 633, 1942. (b) Engel, R. W., and Salmon, W. D. *J Nutrition* **22** 109, 1941. (c) Gyorgy, P., and Goldblatt, H. *J Exper Med* **72** 1, 1946. (d) Hartroft, W. S. *Brit J Exper Path* **6** 483, 1948.

2 Griffith, W. H., and Wade, N. J. (a) *J Biol Chem* **131** 567, 1939. (b) **132** 627, 1940.

3 Hartroft, W. S., and Best, C. H. *Brit M J* **1** 424, 1949.



Figures 1-4

(See legends on opposite page)

lary ischemia which induced the necrosis of tubular epithelium. In chronic experiments with 150 Gm rats, however, sudanophilic droplets were not so numerous and renal cortical ischemia and focal necrosis of the epithelium did not occur^{1d}. It would appear, therefore, that other factors are responsible for the renal destruction which may be produced by chronic (six months or longer) choline deficiency in weanling rats.

Copeland and Salmon⁴ reported the production of neoplasms in rats fed choline-deficient diets from eight to sixteen months. Erickson and Goebbel⁵ corroborated this observation in a smaller percentage of their animals and also observed numerous collections of eosinophilic casts with tubular dilatation and fibrosis in the kidneys of a majority of their rats. Since their interest was primarily in the influence of chronic choline deficiency on tumor production, the renal changes were not described in detail in their preliminary report. The present paper describes the extensive alterations of the renal parenchyma as they appeared at intervals in animals subjected to a choline deficiency for many months.

METHOD

Hooded rats were obtained from the Laboratory of Animal Nutrition, Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn, Ala., Sprague-Dawley rats were secured from the Holtzman Laboratory Animals, Incorporated,

4 Copeland, D. H., and Salmon, W. D. *Am J Path* **22** 1059, 1946

5 Erickson, C. C., and Goebbel, W. *Federation Proc* **8** 354, 1949

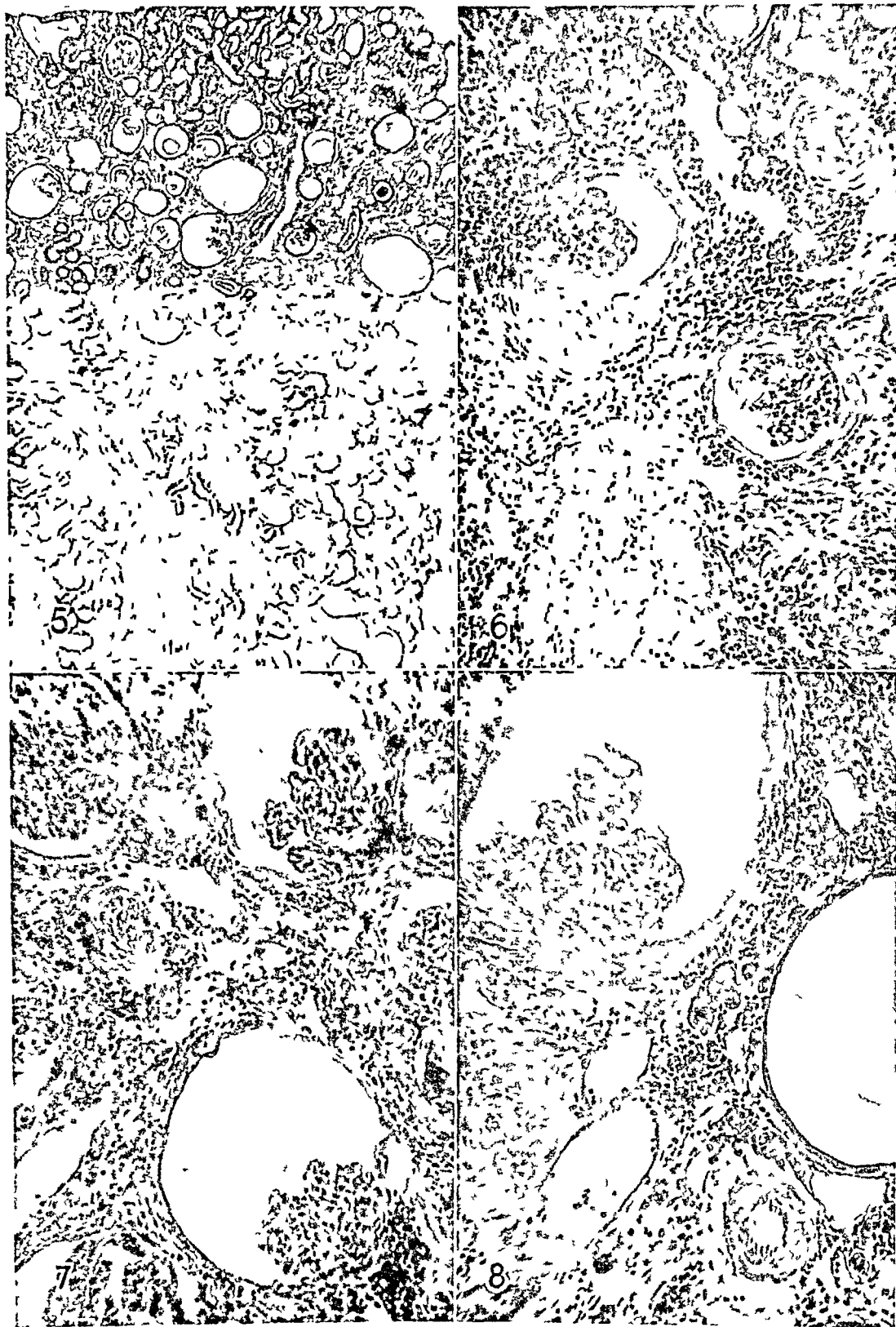
EXPLANATION OF FIGS 1-4

Fig 1—Control rat 2 was fed the control diet for ten months. There is calcification at the corticomedullary junction. The glomeruli and the tubules are normal. The lumens of the tubules are empty. Hematoxylin and eosin stain, $\times 25$.

Fig 2—Control rat 7 was fed the control diet for fourteen months. The glomeruli and the tubules were considered normal. There is an area of calcification, which is limited principally to the corticomedullary zone. There is an eosinophilic granular material in some tubules, however, tubular dilatation was not observed. Hematoxylin and eosin stain, $\times 25$.

Fig 3—Test rat 10 was maintained on a choline-deficient diet for seven and a half months. There was 2 plus destruction of the cortical elements. In this field there are collections of acidophilic casts and tubular dilatation with atrophy of epithelial elements. Bowman's spaces are dilated, some of these contain eosinophilic material. Note the compression of the glomerular tufts where eosinophilic material fills Bowman's spaces. In this field there are also normal-appearing glomeruli. Fibrosis and lymphocytic infiltration are most pronounced near the corticomedullary junction. Only a few of the tubules have undergone calcification in this field. Hematoxylin and eosin stain, $\times 25$.

Fig 4—Test rat 20 was on a choline-deficient diet for thirteen months. The cortical destruction was considered 2 plus. In the section shown the cast collection and subsequent tubular dilatation are pronounced. Compression of glomerular tufts is focal in nature, and it is associated with swelling of Bowman's capsules and the presence of eosinophilic material in Bowman's spaces. There is minimal calcification. The interstitial fibrosis and lymphocytic infiltration are not as prominent as in the previous figure. Hematoxylin and eosin stain, $\times 25$.



Figures 5-8

(See legends on opposite page)

of Madison, Wis. These animals were bred in the McArdle Memorial Laboratory for Cancer Research of the University of Wisconsin and were fed the same stock diet as was used by Copeland and Salmon⁴. The stock diet contained ground wheat, 60.5, meat scraps, 9.4, alfalfa leaf meal, 1.9, iodized salt, 0.5, black strap molasses, 4.7, whole milk powder, 5, crude casein, 12, lard, 5, and cod liver oil, 1 per cent. The experimental diet which was used in this experiment was similar to 43A of Copeland and Salmon⁴. The control rats received diet 43A with 2 Gm of choline added per kilogram of diet.

Rats of both strains were weaned at 23 or 24 days of age. Approximately two thirds of them were fed the low choline and one third the added choline diet. All of the choline-deficient rats were given daily supplements of choline in water by stomach tube during the first week or two to get them over that critical period when they had a great need for choline. The Sprague-Dawley rats were usually given 2 to 4 mg doses and the Alabama rats 4 to 6 mg. By the end of the second week the rats usually gained weight on the diet without choline supplementation. However, they were weighed daily throughout the critical period of the experiment and were given choline whenever a sharp drop in weight indicated the need. Older rats were given 20 to 40 mg doses at such times. When the animals failed to respond to one or two doses of choline supplement, their condition deteriorated rapidly and they died.

Test animals were selected for microscopic study from rats which died or from those which were killed when they became inactive or lost weight rapidly and stopped eating. The majority of the control rats had to be killed, since only a few died with bronchopneumonia. The tissues were fixed in 10 per cent formaldehyde solution and stained with hematoxylin and eosin. A total of 15 control and 23 test rats subjected to choline-deficient diets for periods of six to nineteen months are included in the present study.

RESULTS

Gross Observations—The significant pathologic alterations occurred in the livers, the kidneys and the lungs. Fatty livers with cirrhotic

EXPLANATION OF FIGS 5-8

Fig 5—Test rat 18 was maintained on a choline-deficient diet for twelve months. The tubular and glomerular degeneration was considered to be 3 plus. In this section there are interstitial fibrosis, lymphocytic infiltration and focal calcification. The remaining tubules are clearly dilated and contain eosinophilic casts. The glomerular alterations are advanced, there is compression of tufts, some are small, avascular and cellular, whereas other glomeruli have completely disappeared. Hematoxylin and eosin stain, $\times 25$.

Fig 6—Section of a kidney of the same animal as that in figure 3. There is thickening of Bowman's capsule with destruction of glomeruli. The tubules are replaced by fibrosis and lymphocytic infiltration. Hematoxylin and eosin stain, $\times 150$.

Fig 7—Test rat 14 was on a choline-deficient diet for nine months. The cortical destruction was considered to be 2 plus. Note the definite fibrosis of Bowman's capsules, the eosinophilic material in Bowman's spaces and the variable alterations in the glomeruli. There are also loss of tubules, interstitial fibrosis, lymphocytic infiltration and arteriolar hypertrophy. Hematoxylin and eosin stain, $\times 150$.

Fig 8—Test rat 21 was on a choline-deficient diet for fourteen months. The degeneration of tubules and glomeruli was 3 plus. Note the advanced tubular and glomerular destruction. Fibrosis, lymphocytic infiltration, arteriolar hypertrophy and calcification are also evident. Hematoxylin and eosin stain, $\times 150$.

nodules were observed in the choline-deficient rats. In the control rats almost all of the livers and kidneys were normal on gross inspection. In the deficient group, depending on the severity of renal involvement, there was a progressive increase in size of the kidneys to approximately two times normal. In addition, a fine granularity of the surface with or without retention cysts, was also apparent. The majority of the rats in the control and deficient groups had purulent bronchitis and terminal bronchopneumonia. In over half of these animals these conditions were associated with severe chronic bronchiectasis, cystic dilatation of bronchi and multiple pulmonary abscesses.

Microscopic Observations—Before an analysis of the microscopic pathologic changes is attempted, a description of the progressive tendency of the renal lesions may prove helpful. The most common early observation was the presence of eosinophilic or slightly basophilic staining casts of a homogeneous character. The number as well as the size of these casts varied considerably. Sometimes desquamated epithelial cells made up a part of the cast, these, however, were never a conspicuous feature. Initially there were numerous small casts principally in the distal convoluted tubules and Henle's loops with no or slight evidence of dilatation. Later the casts were five to ten times larger, less numerous, but associated with distinct tubular dilatation. The epithelial cells of the dilated tubules were flattened and atrophic or absent. Initially the tubular obstruction occurred in segments with intervening uninvolved parenchyma, in which the epithelial cells might be swollen and vacuolated. Still later, depending on the extent of the tubular obstruction, the pathologic change became generalized. In such cases there was a reduction in the number of tubular elements, and the remaining tubules were surrounded by connective tissue and foci of lymphocytes. In these sections only isolated granules of hemoglobin pigment were to be observed, either in the epithelial cells of the convoluted tubules or in the phagocytes which had invaded the interstitial space.

The degeneration of the glomerular tufts appeared to be secondary to the tubular obstruction, since most of the compressed tufts occurred in areas of tubular obstruction. Associated with the shrinking of the glomerular tuft there was an extensively dilated Bowman space filled with eosinophilic material. Normal glomeruli and tubules were observed in areas adjoining those just described. Bowman's spaces in which there were no glomerular tufts or in which the glomeruli were undergoing fibrosis were found most frequently in areas of diffuse tubular obstruction and extensive interstitial fibrosis. At this late stage hyalinization and thickening of Bowman's capsule were also a common finding.

Alterations of renal arteries were neither so prominent nor so common as the tubular and glomerular destruction. Intimal calcification

of the larger renal arteries near the hilus was seen infrequently. Hyalinization and hypertrophy of arterioles were observed in over half of the choline-deficient rats. The arteriolar changes, however, were focal in distribution and were present only in areas of glomerular and tubular destruction. The interstitial fibrosis and the lymphocytic infiltration were roughly equivalent to the tubular and glomerular changes. Significant interstitial fibrosis occurred only in the deficient rats, it was not observed in the control group.

Calcification occurred principally in the area of the corticomedullary junction. The calcification appeared to be intratubular and was present in over 90 per cent of the animals. It appeared to be of similar severity and frequency in the test and control groups. Calcification is unusual in control animals, and the cause for it is not evident.

The microscopic data concerning 7 control and 14 test Alabama hooded rats has been assembled in the accompanying table to illustrate more fully the influence of chronic choline deficiency on the renal parenchyma. The extent of involvement was based on the percentage of tubules and glomeruli affected in one section. On this basis the sections examined were separated into four groups. Sections were considered normal when less than 5 per cent of the tubules contained casts and there was no evidence of glomerular destruction. One plus indicates 5 to 20 per cent, 2 plus, 20 to 50 per cent, and 3 plus, 50 per cent or more involvement of the tubular and glomerular elements. The arterial changes, the interstitial fibrosis and the calcification are indicated as being present or absent by a plus or a minus sign.

Examination of the table shows that 1 of 7 control rats exhibited a significant cast accumulation with some glomerular destruction. In the remaining 6 the kidneys were normal except for the presence of calcification in 3. In all of the 14 Alabama hooded rats restricted to a choline-deficient diet for periods of six to fourteen months renal changes were observed. There was 1 plus involvement in 5, 2 plus in 7 and 3 plus in 2. Interstitial fibrosis was not conspicuous until 20 to 50 per cent of the tubules were involved. Arteriolar hyalinization and hypertrophy were more apparent in kidneys with greater destruction.

Observations in both control and deficient Sprague-Dawley rats were in general agreement with those in the Alabama strain. Of 8 control rats maintained eleven to nineteen months, 5 had normal kidneys. There was 1 plus tubular involvement without significant glomerular destruction in 3. Of 9 deficient rats maintained from ten to eighteen months, 3 had normal kidneys. There was 2 plus alteration of tubules and glomeruli in 4 and 1 plus in 2. The microscopic observations suggested that the renal changes were more difficult to produce in the Sprague-Dawley strain.

Since the influence of choline deficiency on the liver has been repeatedly described,⁶ only the frequency of such changes will be given. A diffuse fatty infiltration associated with regenerating liver and discontinuous periportal fibrosis was observed in 18 of 23 test rats and in none of the controls. Confluent periportal fibrosis with greater collections of connective tissue was observed in 12 of 23 rats. Normal livers were found in 16 control and 4 test rats.

Sections of lungs from 30 rats were examined. Moderate to severe bronchiectasis associated with terminal bronchopneumonia occurred in 16 test and 7 control rats. In 7 control rats the lungs were considered normal.

The Effect of Chronic Choline Deficiency on the Kidneys of Alabama Rats

Rat	Diet*	Sex	Time, Mo	Renal Alterations Observed†				
				Casts and Tubular Obstruction	Glomerular Destruction	Interstitial Fibrosis	Arterial Degen- eration	Calcifi- cation
1	C	F	7 5	—	—	—	—	+
2	C	F	10 0	—	—	—	—	+
3	C	M	11 0	—	—	—	—	—
4	C	F	12 0	—	—	—	—	—
5	C	M	12 5	2+	1+	—	—	+
6	C	M	14 0	—	—	—	+	+
7	C	F	14 0	—	—	—	—	—
8	T	M	6 0	1+	—	—	—	+
9	T	M	7 0	1+	—	—	—	+
10	T	M	7 5	2+	2+	+	+	+
11	T	F	7 5	2+	2+	+	+	+
12	T	M	8 0	1+	1+	—	—	—
13	T	M	8 5	1+	1+	—	+	+
14	T	M	9 0	2+	2+	+	—	+
15	T	F	9 5	1+	1+	—	—	+
16	T	M	10 0	2+	3+	+	+	+
17	T	F	11 5	2+	2+	+	—	+
18	T	M	12 0	3+	3+	+	+	+
19	T	F	12 0	2+	2+	+	+	+
20	T	M	13 0	2+	2+	+	+	+
21	T	F	14 0	3+	3+	+	+	+

* C is the control diet, T, the choline deficient diet

† In the first two columns 1+ indicates that 5 to 20 per cent of the tubules and glomeruli were affected in one section, 2+, 20 to 50 per cent, 3+, 50 per cent or more. In the last three columns + or — signifies present or absent as the case may be.

COMMENT

The magnitude of renal destruction observed in this study is in agreement with that observed by Erickson and Goebbel.⁵ The lesions described are more extensive than those observed by Hartroft^{1a} and Hartroft and Best.⁸ This is probably due to the fact that weanling rats were used and the time interval was usually longer in our studies. Vacuolation of epithelial cells of the proximal convoluted tubules, such as is described by Hartroft, was observed in all of the test animals. Recent hemorrhage was not observed in the series. Evidence of previous

6 Engel and Salmon^{1b} Griffith and Wade²

hemorrhages as judged by the presence of hemosiderin in the interstitial tissue was minimal or absent

The renal destruction appeared to be associated with and to follow the accumulation of eosinophilic and basophilic casts in the distal convoluted tubules and Henle's loops. Whether the casts were due to alteration of glomerular permeability with loss of plasma proteins or were associated with elimination of other constituents of plasma is not known, although the former hypothesis seems more plausible. The presence of numerous casts with tubular and glomerular destruction of a segmental character suggests a degenerative rather than an inflammatory lesion. This opinion was further enhanced by a failure to observe neutrophilic cells in the renal pelvis, the collecting tubules or the interstitial tissue of the medulla. In view of the aforementioned findings, the focal collections of lymphocytes in the interstitial spaces were thought to be a sequel of the destruction of tubular and glomerular elements rather than a manifestation of primary chronic inflammatory reaction.

On the basis of our observations it appears that the probable sequence of events was as follows. The initial lesion was vacuolation of epithelial cells of the proximal convoluted tubules. This was followed by the collection of eosinophilic and basophilic casts in the distal convoluted tubules and Henle's loops. The casts eventually assumed such proportions as to cause obstruction of the proximal segment with tubular dilatation. At this stage the tubules resembled thyroid acini, as pointed out by Erickson.⁷ Next, eosinophilic proteinaceous material collected in Bowman's space, dilating the capsule and compressing the glomerular tufts. It is quite possible that protracted intratubular obstruction may be responsible for the hyalinization and fibrosis of Bowman's capsule. Compression of the tufts, thickening of the basement membrane and fibrosis or atrophy and disappearance of glomeruli, leaving empty Bowman's spaces, were observed. The glomerular involvement was so extensive that over 95 per cent exhibited some degree of change. Following the glomerular alterations, focal hyalinization and hypertrophy of arterioles occasionally became evident in the adjacent area.

It seems doubtful whether the present observations have any direct bearing on the pathology of renal disease in man. The observed changes, however, are of sufficient magnitude to account for the hypertension which was first described by Hartroft and Best.³ The renal destruction is probably responsible for losses of substantial quantities of protein in the urine. In these rats, moreover, a profound anemia develops and they apparently become increasingly susceptible to pulmonary infection. For these reasons, then, in future experiments on chronic choline

⁷ Erickson, C. C. Personal communication to authors.

deficiency it must be borne in mind that the renal changes which occur may be responsible for some of the extra-renal changes which are observed in the animals

SUMMARY

A chronic choline deficiency was maintained for as long as six to nineteen months in rats of the Alabama and Sprague-Dawley strains. Twenty-three choline-deficient and 15 control rats that survived six months or longer were studied. The renal alterations were severe. From 20 to more than 50 per cent of the renal parenchyma was destroyed in 13 of 23 deficient rats, whereas only minimal and insignificant changes were observed in the control group. The renal destruction appeared to be associated with and secondary to the accumulation of eosinophilic and basophilic casts which took place in the distal convoluted tubules and in Henle's loops.

Notes and News

Legal Medicine Program Established in Louisiana—At Tulane University of Louisiana, New Orleans, an effective integration of the fields of law and medicine is under way on the graduate and undergraduate levels. Dr. Hubert Winston Smith, former professor of legal medicine at the University of Illinois, Chicago, has been named research professor of law and medicine. He will serve also as professor of law and professor of legal medicine in the college of law and the school of medicine, respectively. New courses and seminars will be devoted to more complete training of trial lawyers in the science of proof and of handling personal injury litigation and in legal psychiatry. Long range plans call for projected developments in clinical legal medicine, legal pathology and scientific crime prevention and detection. A course in legal medicine has been established. It is planned also to offer a course in the medicolegal aspects of behavior problems.

During the past few years Dr. Smith has joined with other specialists in research aimed at reformulating medical sciences in terms of their legal applications and utility. The result has been that more than one hundred and twenty-five studies have appeared in both medical and legal journals. During World War II Dr. Smith served as officer in charge of the legal medicine branch of the Bureau of Medicine and Surgery, United States Navy.

George Minot Lectureship Established—Officers of the Section on Experimental Medicine and Therapeutics of the American Medical Association, desiring to honor, by establishing a "name lecture," an American investigator who has made an exceptional contribution to the development of clinical investigation and therapeutics, suggested at the Interim Session of the Association in St. Louis last November that immediate steps be taken to obtain approval of the idea from the Council on Scientific Assembly and that the lecture be named in honor of Dr. George Richard Minot, Boston, whose contributions to medical knowledge of the causes and methods of control of pernicious anemia have been recognized throughout the world. Both suggestions were unanimously supported by the executive committee of the Section, and approval has been obtained from the Council on Scientific Assembly. A committee of former chairmen of the Section—Drs. Edgar van Nuys Allen, Rochester, Minn., Walter Bauer, Boston, and Carl Dragstedt, Chicago—has been appointed to work out details. The first lecture will be given either at the 1950 or at the 1951 meeting.

Appointments—R. S. Fisher, resident fellow in legal medicine at Harvard Medical School, has been appointed assistant professor of pathology in the medical faculty of Western Reserve University, Cleveland.

Alvan G. Foraker and C. Merrill Whorton have been appointed assistant professors in the department of pathology at Emory University School of Medicine, Atlanta, Ga.

F. W. Sunderman, formerly head of the department of clinical pathology at the Cleveland Clinic Foundation, is now at the head of the department of clinical pathology in the University of Texas Postgraduate School of Medicine.

Philip Hench, of the Mayo Clinic, has been appointed chairman of the Arthritis and Rheumatism Study Section of the United States Public Health Service, National Institutes of Health. The other members are Walter Bauer, of Boston, Granville A. Bennett, of the University of Illinois School of Medicine, Jerome W. Conn, of the University of Michigan, W. Paul Holbrook, of Tucson, Ariz., Robert Loeb, of New York, F. J. Moore, of the University of Southern California School of Medicine, Jane A. Russell, of Yale University School of Medicine, Emil L.

Smith, of the University of Utah School of Medicine, and Alfred L. Wilds, University of Wisconsin

William McDowell Hammon, assistant director of the Hooper Foundation, University of California, has been appointed professor and head of the department of epidemiology at the University of Pittsburgh Graduate School of Public Health. In this position Dr. Hammon will also be responsible for the instructional and research interests of the school in the field of microbiology. His appointment is effective Feb. 1, 1950.

New York Medical College has promoted Francis D. Speer, assistant professor of pathology to professor of pathology and clinical pathologist and director of the department. He succeeds George K. Higgins, who has resigned.

Bela Halpert, formerly director of laboratories at the University of Oklahoma Hospitals and professor of clinical pathology in the University of Oklahoma School of Medicine, has been appointed chief of laboratory service at the Veterans Administration Hospital at Houston, Texas, and associate professor of pathology at Baylor University College of Medicine, effective Oct. 1, 1949.

Fund for Research in the Field of Lymphatic Leukemia—The National Research Council announces that the estate of Charles R. Blakely has donated \$25,000 for the support of research bearing on lymphatic leukemia. Applications for grants are now being entertained. Further information and application forms may be secured from the Chairman of the Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, N. W., Washington 25, D. C.

Fellowships—The National Research Council of Canada will offer thirty-one postdoctorate fellowships for the year 1950-1951, eighteen of which will be awarded in chemistry, three in atomic energy research and ten in physics. The stipend of \$2,820 per annum is supplemented by travel grants to successful candidates from abroad. While appointments in the atomic energy project are restricted to Canadian citizens and British subjects, applicants of all nationalities are welcome in the chemistry and physics divisions. Application forms and further information may be obtained from The Secretary, Laboratories Awards Committee, National Research Council, Ottawa. Applications should be received not later than Feb. 15, 1950.

Donald B. McMullen, associate professor of preventive medicine and public health, University of Oklahoma Medical School, has returned after twenty-six months' leave of absence. Dr. McMullen has been working on the epidemiology and control of schistosomiasis japonica in Tokyo, where he has been senior parasitologist with the 406th Medical General Laboratory.

Deaths—Ralph R. Parker, 61, entomologist, died of a heart attack at his home in Hamilton, Mont., September 4. Director of the Rocky Mountain Laboratory of the National Institute of Health, Dr. Parker was co-discoverer of a vaccine for Rocky Mountain spotted fever.

Dr. William R. Bonnelle, of Fort Wayne, Ind., died May 26, aged 75, of arteriosclerotic heart disease and diabetes mellitus. Dr. Bonnelle was a charter member of the American Society of Clinical Pathologists, one of the founders of the Indiana State Pathological Association, member of the College of American Pathologists, American Association for the Advancement of Science, American Heart Association and Fort Wayne Academy of Science. He served in France and Germany during World War I and was a member of the Indiana Medical Advisory Board no. 4, Selective Service System in World War II.

Dr. Giulio Andrea Pari, who occupied the chair of medical pathology in Padua University, Italy, since 1925, has died at the age of 69. He was a productive investigator, especially in the physiology of neural centers.

Samuel Harold Gray, St Louis, died in Cameron, Wis, August 18, aged 52. Dr Gray was associate professor of pathology at Washington University School of Medicine. He was a specialist certified by the American Board of Pathology and a member of the American Association of Pathologists and Bacteriologists, the College of American Pathologists, the American Society for Experimental Pathology and the American Society of Clinical Pathologists. He was affiliated with the Jewish Hospital and served during World War II.

John Joseph Larkin Jr, Boston, died in Sterling, Mass, August 26, aged 35, of accidental drowning. He was a member of the American Medical Association and the College of American Pathologists, a specialist certified by the American Board of Pathology, assistant professor of pathology at Tufts College Medical School, pathologist at the Holy Ghost Hospital in Cambridge, where he was in charge of cancer research, and St Elizabeth's Hospital. He served in World War II from 1942 to 1945 as captain, later major, at Base Hospital 136 in England and France.

William Henry Watters, 73, pathologist, died October 10 in Hyannis, Mass. Dr Watters was at one time professor of pathology at Boston University and for many years had been an associate in the legal department of the Harvard University Medical School.

Society News—At the meeting of the Wisconsin Society of Pathologists in Milwaukee on October 5, the following officers were elected for the ensuing year: president, W A D Anderson, Milwaukee, vice president, Walter H Jaeschke, Madison, secretary-treasurer, Robert S Haukohl, Milwaukee, and board of censors, J B Miale, Marshfield.

Awards—At the annual meeting of the American Cancer Society, Bowman C Crowell, Chicago, was awarded the society's 1949 medal "in recognition of his outstanding contributions to the control of cancer." Clifford Calvin Nesselrode, retiring president of the society who presented the award, praised Dr Crowell's "signal accomplishments in developing and extending improved services to cancer patients through diagnostic and treatment clinics." Dr Crowell recently retired from his position as associate director of the American College of Surgeons.

Cancer Research Fellowships—The British-American Exchange Fellowships in Cancer Research, awarded by the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, are now open to citizens of the United States who have M D, S D or Ph D degrees. Fellowships are awarded for one year and carry a stipend of \$4,020 and an allowance of \$600 for travel to the place of the fellowship in Great Britain. Application forms may be secured and submitted to the Executive Secretary, Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D C.

Excerpta Medica—*Excerpta Medica*, a monthly abstracting service drawing on articles in every available medical journal in the world, was started in 1947 by the government of the Netherlands. It is now organized on a "not for profit" basis. The board of chief editors consists of M W Woerdeman, chairman, Morris Fishbein and W P C Zeeman.

As soon as the *Quarterly Cumulative Index Medicus*, published by the American Medical Association, is brought up to date, its editors hope to indicate in the index references to abstracts that will be easily available in *Excerpta Medica*. *Excerpta Medica* is published in fifteen sections, each of which may be subscribed to separately. Section 5 is on general pathology and pathologic anatomy.

Books Received

HISTOLOGIE UND MIKROSKOPISCHE ANATOMIE DES MENSCHEN I ZELLEN-UND GEWEBELEHRE By Wolfgang Bargmann, M D, professor of anatomy, University of Kiel, Germany Pp 210, with 192 text figures and 1 colored plate Stuttgart, Germany Georg Thieme, 1948

On the surface this excellent first volume of a textbook of histology does not reveal the difficulties which beset scientists, writers and publishers in present day Germany, for the quality of its paper and printing and the clarity of the illustrations (including photomicrographs) measure up to the traditional high merit of German book making But because of the loss, during the war, of the author's collection of preparations and figures, only the cooperation of his colleagues at other universities, and finally the energy of the publisher, Dr Bruno Hauff, made possible the appearance of the book

Dr Bargmann, a pupil of von Mollendorff, has succeeded in his endeavor to present to the student a more modern approach to the study of microscopic anatomy, that is, instead of restricting himself to the usual descriptions of static form, he gives a conception of the dynamic changes of the microscopically visible structures on the basis of their submicroscopic construction

The first part of the book, dealing with cytology—in the broader sense, with living matter, since it includes intercellular substance—occupies 65 pages In this short space the author has done an admirable piece of writing He introduces the beginner clearly to the different methods of studying living as well as fixed and prepared material He brings the information up to date, mentioning (1) histochemical and quantitative methods, (2) ultraviolet, polarization, fluorescence, roentgen phase and electron microscopy and (3) the use of supersonic waves to divide matter into smallest fragments and so permit investigation to advance into the molecular dimensions of the realm of form Under the caption "The Cell" he takes up (1) the structure of the nucleus and its parts, (2) the cytoplasm, its organoids, its paraplasm (cell inclusions—granules, vacuoles, crystals, etc—of lipids and lipochromes, glycogen, proteins, melanins, etc), its metaplasm, the formative basis of specific functions (neurofibrils, myofibrils, tonofibrils, etc) and its hyaloplasm, or fluid substrate, (3) ultrastructure of the living mass, 8 pages being given to its discussion, (4) cell growth and multiplication (mitosis, meiosis and amitosis), (5) functional morphology of cells (mechanics, kinetics, metabolism, irritability), (6) aging and death of the cell Throughout the book one never loses sight of the bridge between morphology and structural chemistry and physiology

The second part of the book presents the epithelial tissues, the connective and supporting tissues, the muscular tissues and the nervous tissues Any classification is, of course, arbitrary, and that of the fundamental tissues becomes a bed of Procrustes, irrespective of which point of view—genetic, structural or functional—one chooses as a basis of systematization But that Bargmann, to escape part of the dilemma, should relegate the discussion of blood and lymph to the chapter on the circulatory system (to appear in volume 2, on microscopic anatomy or organology) is like cleaving through the Gordian knot, if the reader will excuse another simile here He follows the classification of Kolliker (*Handbuch der*

Gewebelehre des Menschen, Leipzig, W. Engelmann, 1855), who listed the four groups of tissues just indicated. Studnicka (*Allgemeine mikroskopische Anatomie und Organization der lebendigen Masse*, in von Mollendorff, Wilhelm, editor, *Handbuch der mikroskopischen Anatomie des Menschen*, Vienna, Julius Springer, 1929, vol 1, pt 1) placed blood and lymph in a fifth group, a classification with which most histologists agree. But Maximow (in von Mollendorff's *Handbuch*, 1927, vol 2, pt 1) classified the blood forming and destroying tissues with the connective tissues, and Bloom (in Maximow, A. A., and Bloom, W. *Textbook of Histology*, ed 5, Philadelphia, W. B. Saunders Company, 1948) put the fully formed blood and lymph there. This solution seems even more far fetched than Bargmann's. Despite the fact that in morphology categoric lines cannot be drawn perfectly, the functional distinctions between blood and lymph and their cells and the connective tissues are sufficiently definite to warrant separate grouping. (Incidentally, the reviewer has always wondered why histologists have failed to recognize germinal, or better procreative, that is, spermiogenic and oogenic, tissues as a sixth—really the first—group of fundamental tissues.)

Bargmann's book is to be recommended to medical students. Even investigators who are working with histologic methods in physiology, pharmacology, surgery and other fields will find it stimulating. Its style is lucid, concise and orderly, and many of the numerous illustrations are original. A combined name and subject index facilitates its use as a textbook.

TUMORS OF BONE Charles F. Geschickter, M.D., professor of pathology, Georgetown University Medical School, consultant in pathology, United States Naval Medical School, consultant in pathology, Mt. Alto Veterans Administration Hospital, pathologist-in-chief, Gallinger Municipal Hospital, Washington, D. C., and Murray M. Copeland, M.D., professor of oncology, Georgetown University Medical School, consultant in surgery, Gallinger Municipal Hospital, Washington, D. C., special consultant, Federal Security Agency, Public Health Service, Cancer Control Branch, Washington, D. C. Third edition. Pp 810, with 642 illustrations. Price, \$17.50. Philadelphia, London and Montreal: J. B. Lippincott Company, 1949.

This book is the product of a wide survey of the literature of bone tumors and allied conditions and a study of a large clinical material, much of which has been followed over long periods. Special emphasis has been given to gross and microscopic pathologic investigation and its correlation with roentgenologic and other diagnostic procedures in the care of the patient. Long term follow-up studies have been made of the results of various forms of treatment.

In view of the general excellence of these sections it is a question whether some other sections, such as the introductory one on interpretation of clinical findings and endocrinopathies and rare diseases of bone, might better have been deleted and space allowed to a more lengthy and detailed discussion of the surgery of bone tumors. Under cancer, more might have been said about the level of amputation and the removal of regional lymph nodes. There is no discussion of extensive resection and bone transplantation for selected cases of bone sarcoma. Nonoperative therapy, including the use of roentgen radiation, radium, isotopes, androgen and estrogen, nitrogen mustards and urethane, are concisely discussed, with the results to be expected from their use in different types of tumors.

In any classification of bone tumors defects will inevitably persist until more is known about the etiologic factors. In general, the gross and microscopic

descriptions of the tumors reported, both cancerous and noncancerous are of a high order, but exception may be taken to the nomenclature employed for some of them. The noncancerous tumors are well classified according to tissue or cell type, but the same procedure is not followed for the cancerous tumors. Instead, their classifications follow Ewing in large measure, who stated that all cases of sarcoma beginning in bone, whether or not the sarcoma comes from bone-forming cells, should be classified as cases of osteogenic sarcoma, but who did not adhere strictly to the rule himself. Thus cancers derived from bone-forming cells are designated as osteogenic sarcoma—osteoblastic or sclerosing type. But cancerous cartilaginous tumors are of three types, osteogenic sarcoma—primary chondrosarcoma, osteogenic sarcoma—secondary chondrosarcoma, and chondroblastic sarcoma.

Tumors generally designated as angiosarcoma or malignant bone aneurysm have been classified as "osteolytic osteogenic sarcoma" because the observers considered them to consist of cancerous forms of plump spindle cells and pleomorphic osteoblasts. An attempt is also made to place some tumors rich in giant cells in this class. These are ill advised changes, as the term gives no clue to the histologic aspect of the tumor and in all instances sarcoma is more or less osteolytic or bone absorbing. Fibrosarcoma, which usually produces extensive central erosion of bone, is considered always to be of periosteal origin, a finding not substantiated by the great majority of experienced pathologists. Ewing's sarcoma or endothelial myeloma begins in the bone, but despite this fact, it is not classified as a type of osteogenic sarcoma. Neither is it considered an endothelioma, as Ewing held. Ewing's tumor is thought to originate most likely from the reticulum cells lining the sinuses of lymphoid tissue and giving rise to cells of the lymphocytic series. There is no discussion of reticulum cell sarcoma as described by Parker and Jackson, but reference is made to the fact that bone metastases of neuroblastoma are frequently diagnosed incorrectly as Ewing's sarcoma. In order to avoid the confusion created by this nomenclature, it would be better to drop the term "osteolytic osteogenic sarcoma," as well as "Ewing's tumor," which is now recognized as designating not an entity but various round cell sarcomas, also to limit "osteogenic sarcoma" to the cancers arising from cells of osteoblastic origin, and to classify sarcomas, as well as benign tumors, of bone according to tissue or cell type.

A worth while contribution has been made in recognizing periosteal osteoma as an entity. It has usually been mistaken for sarcoma and wrongly treated by amputation instead of by wide excision.

There are many valuable sections on tumors of regions of the skeleton and on allied conditions which must be differentiated from bone tumors.

The book will prove useful to pathologists, orthopedic and general surgeons and internists.

STUDIES IN AIR HYGIENE By R B Bourdillon, O M Lidwell and J E Lovelock, with W C Cawston, L Colebrook, F P Ellis, M Van Den Ende, R E Glover, A M MacFarlan, A A Miles, W F Raymond, E Schuster and J C Thomas. Medical Research Council Special Report Series no 262. Pp 356. Price 7s 6d. London, England. His Majesty's Stationery Office, 1948.

The fear that World War II might be associated with great epidemics of influenza and other "air-borne" infections led to the organization, in London, in 1940, of a team of investigators under the direction of the late Sir Patrick Laidlaw to explore new means of air hygiene. After Laidlaw's death the direction of the work fell to Dr C H Andrewes and later to Dr R B Bourdillon. Report 262.

which the Medical Research Council justifiably calls "a landmark in the study of air hygiene," is a collection of forty-four papers by members of the group, plus an appendix dealing with certain technical aspects of the work

The first twelve papers deal with the problem of air sampling. The ingenious and useful "slit sampler" invented by Dr. Bourdillon provides the background for most of these papers. Theoretic and practical problems presented and solved by the slit sampler are discussed, and the various "models" of the sampler are described. Though the slit sampler has not been employed in work on air-borne infection in the United States, there is no doubt that it is one of the most important tools so far developed in this field. Its particular merit is that it permits the bacteriologic content of the air to be measured continuously and accurately before, during and after the use of bactericidal agents.

"Air Disinfection by Chemicals" is the title of the second section of the report. Herein are described the experiments carried on in the search for a chemical agent which would fulfil the rigid requirements of a "perfect" air sterilizing agent. Though the ideal compound has not yet been found, much progress has been made. The British and American workers are in agreement that the action of the chemicals advanced to date, which are effective against air-borne micro-organisms in concentrations of micrograms or even fractions of a microgram per liter of air, occurs when the chemical in vapor phase condenses on the air-borne particle. Thus, it is incorrect to speak of these chemicals as "aerosols." In the United States, propylene and triethylene glycol have been the subjects of most of the investigations of bactericidal vapors. Bourdillon and his group explored the properties of a long list of compounds, of which lactic acid and alpha-hydroxy-alpha-methyl butyric acid are the most promising. Both appear to be active against air-borne bacteria carried in dry particles, a property not shared by many "air-sterilizing" agents.

Studies with ultraviolet rays attracted some attention. The limitation placed on the usefulness of this method by the fact that the radiation has to be restricted to the upper third of a room because of danger to the eyes is well recognized. Other methods of air disinfection studied included the use of heat (especially for the air of laboratories dealing with dangerous pathogens), the passage of air through filters and the use of masks.

Dr. Bourdillon and his group appreciate the great difficulties which lie in the way of determining just how many infections of the respiratory tract are strictly air borne. However, they rightly point out that there is much justification for attempting to reduce the load of air-borne micro-organisms by the methods now at our disposal. Though no data concerning the infecting dose of respiratory pathogens for human beings are available, animal experiments have shown that both the severity and the mortality of air-borne infections are directly influenced by the dosage of virus or bacteria. They discuss also the argument that reduction of the concentration of air-borne bacteria is a bad thing because it will ultimately impair human immunity to these pathogens. They do not hold with this argument and point to the perhaps debatable analogy with water hygiene to support their contention. This reviewer is in accord with their general conclusions. Though in the case of such infections as measles and chickenpox it is advisable for children to acquire immunity by having the clinical disease before they are fully grown, there is no evidence that this principle applies to common respiratory pathogens. Furthermore, no means of air disinfection is likely ever to accomplish 100 per cent reduction of the number of air-borne micro-organisms in all places where human beings meet.

TEXTBOOK OF HISTOLOGY By Jose F. Nomidez, D.Sc., late professor of anatomy, Cornell University, and professor of microscopic anatomy, University of Georgia, and William F. Windle, Ph.D., Sc.D., professor of anatomy, University of Pennsylvania. Pp. 456, with 287 illustrations (209 drawings and diagrams, and 193 photomicrographs). Price \$6.75. New York: McGraw-Hill Book Company, Inc., 1949.

Professor Nomidez had been working for some time on the illustrations and manuscript of a textbook of histology when he died in the autumn of 1947, only a few weeks after arriving at the University of Georgia. Some weeks later Professor Windle took on the task of completing the work as nearly as possible according to the plan laid down by Nomidez, who had stated his objective in the following way: "The purpose of the proposed book is to present in concise form the fundamental facts on the finer structure of the mammalian body, including man, and to emphasize as far as possible the functional aspects." It was soon realized that a book with a concise text would have to be adequately illustrated and that figures would fit the text better if they were drawn for the purpose instead of being borrowed from other textbooks and scientific journals. In preparing the text, it has been borne in mind that the inclusion of controversial subjects, names of authors and references make reading difficult. Many students lack proper preparation for the study of histology. The situation has been taken into account, and little knowledge of anatomy and physiology is taken for granted.

Accordingly, the textbook is meant for the beginner. It is not a reference work, for not only are many details lacking but also a comprehensive bibliography. In the appendix Windle has listed 18 histologic, embryologic and physiologic textbooks in English which he used in the preparation of this book and to which he directs the exceptional student for bibliographic information. Too, at the end of each chapter, he gives two or three selected references, which he annotates pertinently in order to guide the interested student to such collateral reading.

Since only one half of the text existed in rough copy at the time of Nomidez's death, Windle found it expedient, for the sake of coherence of description and consistency of style, to rewrite even that part. His method of approach is adapted to the novice. The sentences are short, simple and direct, indeed, frequently they begin with the pronoun "you" or imply it. There is little adornment of phraseology, and only exceptionally is the dramatic element exploited.

The great majority of the 200 drawings and diagrams were made by Nomidez. For the most part they are original, are executed simply and express clearly the points to be illustrated. The equal number of remaining figures are photomicrographs, most of which were taken under Dr. Windle's direction. Many of these are excellent, especially those that were taken under the lower magnifications to serve as orientation pictures in the study of histologic relations and construction. On the other hand, there is a relatively large number—30 or 40—of other photomicrographs, taken under higher magnifications, which are unsatisfactory because of poor focus and lack of definition and contrast. These are more confusing than helpful to the beginner. Doubtlessly, better ones will take their place in a second edition. Windle is to be commended for stating in every case the magnification employed in the preparation of the figure.

Windle is in accord with all alert teachers of cytology and histology regarding the desirability of making the subject come alive. Few histologic laboratories at present are equipped to set up *in vitro* or *in vivo* experiments for the benefit of the beginning student. Hence, increasingly greater use is made of motion pictures for conveying to him the concepts of living cells and tissues. To assist the student

and the teacher in the selection of suitable motion pictures to supplement the lectures and the laboratory work, Windle has appended to his textbook a reference list of films and their sources

The Nomdez and Windle textbook represents a fine piece of book-making by the publishers, McGraw-Hill. The appearance is pleasing, and the binding, the quality of paper and the printing are excellent. On the whole, it is well edited, and it is notably free of typographic errors. The reviewer believes it will become a popular book with beginning students of histology.

ATLAS OF PERIPHERAL NERVE INJURIES By William R. Lyons, Ph.D., associate professor of anatomy, University of California Medical School, and Barnes Woodhall, M.D., professor of neurosurgery, Duke Medical School, Durham, N.C. Price \$16. Philadelphia: W.B. Saunders Company, 1949.

This handsome volume presents an enormous amount of material, much of it of great interest to pathologists, as well as to anatomists and neurosurgeons. In the foreword, Dr. Glen Spurling comments that the book, a product of World War II, "differs from many other research projects accomplished during the war in that it was supported by no elaborate budget and was achieved by the authors while they were carrying, at the same time, a heavy load of clinical and administrative responsibilities."

The book embodies the experience accumulated by the authors in dealing with peripheral nerve injuries in the Walter Reed and Halloran General Hospitals during 1943 to 1945, supplemented by specimens contributed by other neurosurgeons. In the course of the day to day work some 3,000 photographs and photomicrographs were collected. There are thus presented several hundred photographs, with accompanying text, all portraying peripheral nerve injuries, and including clinical illustrations as well as those of gross and microscopic pathology.

After a brief discussion of terminology and of peripheral nerve structure, as well as of methods of staining and fixation, the remainder of the volume is divided into four sections, dealing respectively with completely severed nerves, traumatic nerve lesions without loss of continuity, nerve sutures and nerve grafts. In each section there is a relatively brief introduction, followed by many dozen fine photographs and photomicrographs. There are 135 pages of plates, each with a facing page of descriptive legends, so that illustrations and legends together occupy 270 of the book's 331 pages.

A question may well be raised regarding the wisdom of the mode of presentation. The texts are relatively brief and do not furnish any comprehensive analysis of the subjects treated, although 195 bibliographic references are made. The photographs are excellent, but there is a certain repetitiveness which should not obtain in an atlas. There are many illustrative cases, with data on the clinical course, and photographs of gross and microscopic specimens. These are of great concrete value and interest, more so, perhaps, than the array of photographs exemplifying general changes. Among the latter, however, there are many fine illustrations which will be greatly appreciated by all pathologists. Many of the plates are in color, adding materially to the cost of the book without compensatory enhancement of its scientific worth.

The sections on nerve suture and nerve grafts are of especial interest, but the texts are far too brief. It appears to this reviewer that if, in the entire book, the illustrations were reduced to a quarter of their number and the text expanded tenfold, the volume would be of greater value. But then, perhaps, it would no longer be an atlas.

PATHOLOGISCHE PHYSIOLOGIE DER FRISCHEN, GESCHLOSSENEN HIRNVERLETZUNG, INSBESONDERE DER HIRNERSCHÜTTERUNG, KLINISCHE, ANATOMISCHE UND EXPERIMENTELLE BEFUNDE NEBST ANHANG THERAPEUTISCHE FOLGERUNGEN By R. Wanke, professor of surgery at the University of Kiel Pp 196, with 90 illustrations Stuttgart Georg Thieme Verlag, 1948

The first section of this monograph deals principally with experimental and clinical observations made in cases of acute cerebral concussion. The number of animal experiments is limited, and the experimental work is not thorough. The author has attempted to prove that many of the phenomena of cerebral concussion may be referred to a disturbance of the autonomic nervous system due to involvement of autonomic centers of the midbrain and neural pathways connected with these centers. The evidence offered in proof of this thesis is not convincing.

A second section of the monograph is concerned with cerebral edema, intracerebral hemorrhage and changes of cerebrospinal fluid pressure occurring in patients with acute cerebral contusion or concussion. The observations here are generally in accord with those which have been well recognized for some time.

The final section of the monograph deals with the treatment of the patients and the prognosis. Except for necessary measures in handling complications, the conclusion is reached that the best method of treatment is that of noninterference.

This volume adds little to the existing body of information about cerebral concussion, its complications and method of treatment. In some respects, the data add further confusion, because this author, as well as most others, has failed to recognize that a critical approach to an analysis of the causes of the disturbances following acute cerebral injury and a proper evaluation of treatment should be conducted by methods which are capable of reproducing, qualitatively and quantitatively, acute cerebral injury, either of local or of generalized distribution.

THE 1948 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY Edited by Howard T. Karsner, M.D., professor of pathology and director of the Institute of Pathology, Western Reserve University, Cleveland, assisted by Herbert Z. Lund, M.D., assistant professor of pathology, Western Reserve University, Cleveland. *Clinical Pathology* Edited by Arthur Hawley Sanford, M.D., professor of clinical pathology, University of Minnesota (Mayo Foundation), and senior consultant, Division of Clinical Laboratories, Mayo Clinic. Pp 538, with 127 illustrations. Price \$4.50. Chicago: The Year Book Publishers, 1949.

This addition to the series of Year Books presents an adequate survey of the fields of pathology and clinical pathology and will be valuable not only to pathologists but to internists, surgeons and others as well. A notable feature, included in the section on pathology, is a short review of articles which give a good synthesis of recent work on shock, cytologic diagnosis of tumors, vitamin B₁₂, pulmonary berylliosis and iron metabolism. These articles are most helpful and could be increased in future editions. The articles selected for inclusion are well chosen and the abstracts are of sufficient length to do justice to the original. The contents of the book are too numerous to be mentioned in this review, and it is hoped that this will induce others to read it for themselves. They will be richly rewarded.

HISTOLOGIA DE LARINGE, TRAQUEA, BRONQUIOS Y PULMONES By Manuel Perea Muñoz, profesor adjunto de anatomía patológica y jefe del laboratorio de anatomía patológica del Instituto de Fisiología de la Facultad de Ciencias Médicas de Córdoba. Pp 59, with 12 illustrations. Buenos Aires, Argentina: Ediciones Aguamarina, 1948.

UBER NEUROME UND NEUROFIBROMATOSE, NACH UNTERSUCHUNGEN AM MENSCHLICHEN MAGENDARMSCHIAUCH By F Feyrter, O professor der Pathologie, Georg Hanusch-Krankenhaus der Wiener Gebietskrankenkasse fur Arbeiter und Angestellte, Wien XLV Pp 125, with 37 illustrations and 6 tables Price, \$3 50 Vienna, Austria Verlag Wilhelm Maudrich (New York Grune & Stratton, Inc) 1948

The book is divided into four sections and deals with neuroma, Recklinghausen's neurofibromatosis and fibroma of the gastrointestinal tract In section 1, the types of neuroma are given as follows (a) the fusiform, (b) the multiform, (c) the microcytic, (d) the reticular, (e) the granular, (f) the myoneuroma and (g) the macrocytic type They are distinguished from the myoma They arise from or in the region of the Meissner and Auerbach plexuses The sites and the frequency of occurrence are discussed Frequently neuroma occurs in the stomach and the small intestine The fusiform type is the most common, being found in three fourths of all cases

Section 2 discusses Recklinghausen's neurofibromatosis of the gastrointestinal tract These tumors are derived from the endoperineural tissue and appear with and without cutaneous neurofibroma They are found in the small intestine, the large intestine and the rectum and are polypoid or plate-shaped They are composed of ganglion cells, satellite cells, Schwann's cells and endoperineural cells Some are associated with capillary hemangioma

Fibroma of the gastrointestinal tract is discussed in section 3 It invariably occurs in patients older than 45 years of age It is found in the ileum in submucous position and is more frequent in men than in women It arises from the perineural tissue

In section 4 certain glandular polyps of the gastric pylorus are discussed, which have an interstitial origin from endoperineural covering and nerve network Growths of this sort are restricted to that portion of the mucous membrane in which the neurogenous Meissner plexus is developed One type is composed of acellular fine fibers without many capillaries, and the second type is composed of large fibers and has a vascular appearance

The book expresses the author's views based on his experiences

A YEAR WITH OSLER 1896-1897 NOTES TAKEN AT HIS CLINICS IN THE JOHNS HOPKINS HOSPITAL By Joseph H Pratt, a Member of the Class of 1898 Cloth Price, \$4 Pp 209, with 6 illustrations Baltimore Johns Hopkins Press, 1949

Osler and his clinical work in 1896-1897 are well described in the introduction and preface The photograph of Osler at that time and the five plates are good and highly interesting The "clinical notes" (198 pages) reproduce accurately clinical talks and expositions by Osler The book is a valuable record "not only of a great teacher, but of great teaching"

CLINICAL CHEMISTRY IN PRACTICAL MEDICINE By C P Stewart, M Sc (Dunelm), Ph D (Edin), Reader in Clinical Chemistry, University of Edinburgh, Senior Biochemist, Royal Infirmary, Edinburgh, and D M Dunlop, B A (Oxon), M D, F R C P (Edin), F R C P (Lond), Christison Professor of Therapeutic and Clinical Medicine, University of Edinburgh, Physician, Royal Infirmary, Edinburgh Third edition Price \$5 Pp 324, with 30 illustrations Baltimore The Williams and Wilkins Company, 1949

One of the purposes of this book is to give information on the circumstances in which a chemical examination may be of service in diagnosis and prognosis of

diseases and in control of therapy. A second purpose is to give an appreciation of the rationale of chemical pathology in its application to practical medicine, though what is meant by "practical" is not made clear. A third purpose is to provide a broad knowledge of how various chemical analyses are carried out and a detailed knowledge of the method of performing many of the simpler tests which do not require much time or equipment and which may be performed in the physician's own surgery or dispensary. The book is addressed to the practitioner, house physician and senior student.

Most of the material in the book will be familiar to all physicians. The group to whom the book is addressed will not be stimulated by its contents, for most of the methods are too complicated and their interpretations too devious. Of the simple tests described, those which may be carried out in a physician's office by the physician or nurse are tests for sugar and protein in the blood, urine and spinal fluid, lactic acid and hydrochloric acid in the gastric juice, the amylase tests for acute pancreatitis, the van den Bergh and the sulfobromophthalein sodium tests for hepatic function and the erythrocyte sedimentation test. Not more than twenty pages would be needed to deal adequately with those tests. The boredom with which a practitioner will read this book is due to its failure to give the rationale of chemical pathology which it sets out to do. Insofar as the book reflects the desires of the chemist, it gives good advice on the collection and preservation of samples for analysis.

STUDIES ON HOOKWORM DISEASE IN SZECHWAN PROVINCE, WEST CHINA
By K Chang and co-workers. American Journal of Hygiene Monographic Series, no 19, May 1949. Supported by the De Lamar Fund of the Johns Hopkins University. Pp 152, illustrated. Price \$3. Baltimore. The Johns Hopkins Press, 1949.

Using the smear technic, the Willis brine flotation technic and the Stoll dilution egg-counting method in the examination of fecal specimens, the authors obtained accurate information on the severity of hookworm disease in the separate areas of the province, the population of which is estimated at 45,000,000. Their findings were then broken down into occupational and age groupings and correlated with existing climatic and agricultural conditions. The conclusions reached point the way toward a clearer understanding of the many factors involved in the treatment and control of hookworm disease. There are numerous graphs and charts which further interpret this exacting biologic study.

CANCER OF THE ESOPHAGUS AND GASTRIC CARDIA. Edited by George T Pack, B S, M D, clinical professor of surgery, New York Medical College, attending surgeon, Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. Pp 192, with 25 illustrations. Price \$5. St Louis. V C Mosby Company, 1949.

This is a reprint of twelve articles on the present treatment of cancer of the esophagus and gastric cardia in the June 1948 number of *Surgery*.

KOSMETIK UND ALLGEMEINE PATHOLOGIE. By Dr Med Franz Halla. Pp 118. Price \$2.50. Vienna, Austria. Verlag Wilhelm Maudrich (imported by Grune & Stratton, New York), 1948.

This booklet deals with the relations between the skin and systemic disease, which the author claims have been neglected in the literature. It does not, however, go into the problems thoroughly.

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